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# CLINICAL and MOLECULAR HEPATOTOLOGY

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**Review**

# Therapeutic mechanisms and beneficial effects of non-antidiabetic drugs in chronic liver diseases

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The global burden of chronic liver disease (CLD) is substantial. Due to the limited indication of and accessibility to antiviral therapy in viral hepatitis and lack of effective pharmacological treatment in nonalcoholic fatty liver disease, the beneficial effects of antidiabetics and non-antidiabetics in clinical practice have been continuously investigated in patients with CLD. In this narrative review, we focused on non-antidiabetic drugs, including ursodeoxycholic acid, silymarin, dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxibiphenyl-2,2'-dicarboxylate, L-ornithine L-aspartate, branched chain amino acids, statin, probiotics, vitamin E, and aspirin, and summarized their beneficial effects in CLD. Based on the antioxidant, anti-inflammatory properties, and regulatory functions in glucose or lipid metabolism, several non-antidiabetic drugs have shown beneficial effects in improving liver histology, aminotransferase level, and metabolic parameters and reducing risks of hepatocellular carcinoma and mortality, without significant safety concerns, in patients with CLD. Although the effect as the centerpiece management in patients with CLD is not robust, the use of these non-antidiabetic drugs might be potentially beneficial as an adjuvant or combined treatment strategy. (*Clin Mol Hepatol* 2022;28:425-472)

**Keywords:** Nonalcoholic fatty liver disease; Viral hepatitis; Cirrhosis; Non-antidiabetic drugs; Treatment

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## INTRODUCTION

Over the last decade, chronic liver disease (CLD) has become a major cause of morbidity and mortality worldwide, accounting for 2 million deaths globally.<sup>1</sup> The most common etiologies of CLD are nonalcoholic fatty liver disease (NAFLD), followed by hepatitis B virus (HBV), hepatitis C virus (HCV), and alcoholic liver disease.<sup>2</sup> Since cirrhosis and hepatocellular carcinoma (HCC) as major complications of CLD contribute to liver-related morbidity and mortality, effective management of patients with CLD is crucial to reduce the forthcoming disease burden and health expenditures.<sup>3</sup>

Although the prevalence of NAFLD is increasing globally and emerging as a major cause of advanced liver diseases, effective and evidence-based pharmacotherapy is still lacking.<sup>4</sup> In viral hepatitis, potent antiviral therapy using nucleot(s)ide analogues and direct-acting antivirals (DAAs) is the mainstay treatment; however, only selected patients are candidates for antiviral therapy, and the supply of antiviral drugs is often limited in under-developed countries.<sup>5,6</sup> In addition, research has shown the combined fatty load in patients with viral hepatitis to have an unfavorable influence on long-term outcomes.<sup>7</sup> Therefore, varying drugs, including antidiabetics, antioxidants, lipid-lowering drugs, probiotics, and anti-platelets, which might have potential beneficial effects, have been continuously investigated in patients with CLD.<sup>8-15</sup> In *in vitro* studies, diverse therapeutic mechanisms of these drugs, including hepatoprotective, antioxidative, anti-inflammatory, and anti-lipogenic properties, have been suggested.<sup>16-27</sup>

Indeed, several clinical studies have determined that these drugs induce histological and biochemical improvements and thus improve long-term outcomes in patients with CLD.<sup>8-15</sup> In addition, several drugs have been found to affect metabolic

parameters, including the anthropometric index, insulin resistance, and lipid profiles.<sup>28-30</sup> In contrast, other studies have suggested that these drugs have no beneficial therapeutic effect in patients with CLD.<sup>8,12,31-34</sup> In this review, we summarized the therapeutic mechanisms and beneficial effects of non-antidiabetic drugs in patients with CLD.

## URSODEOXYCHOLIC ACID (UDCA)

### Mechanism of action

UDCA is a hydrophilic stereoisomer of chenodeoxycholic acid whose efficacy has been proven in primary biliary cholangitis (PBC), making it the recommended first-line treatment in affected patients.<sup>35,36</sup> Experimental models have shown that UDCA increases the secretion of bile acids and other anionic molecules, such as glutathione conjugates or bilirubin glucuronides, which abrogates cholestasis resulting from hydrophobic bile acids, cytokines, or sex hormones.<sup>16,17</sup> One of the mechanisms involved in the increased secretion of bile acids is the upregulation of hepatobiliary transporter genes such as bile salt export pump and multidrug-resistance proteins 2 and 3 (Fig. 1).<sup>37</sup>

UDCA alleviates intracellular oxidative stress via various mechanisms. Nuclear factor erythroid 2-related factor 2 (NRF2) is a critical stress sensor and a key transcription factor for detoxification, and UDCA enhances NRF2-mediated hepatocellular antioxidative processes in the rat liver.<sup>38</sup> UDCA was also shown to normalize excessive myeloperoxidase activity and reactive oxygen species (ROS) production in stressed rat livers by enhancing the intracellular levels of a reduced form of glutathione.<sup>39</sup> In liver-derived cell lines, the intracellular ROS levels were shown to be increased by palmitate treat-

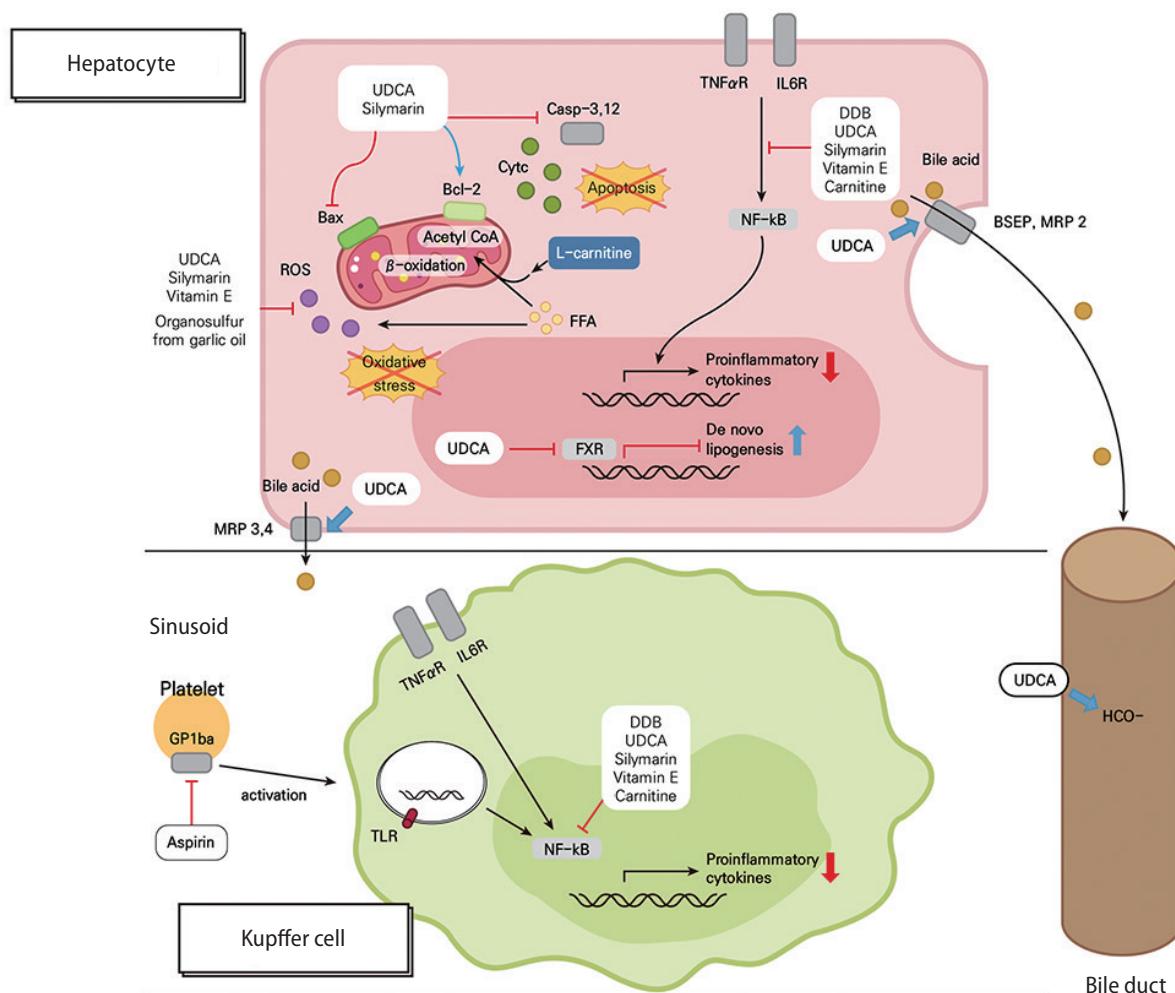
### Abbreviations:

ALT, alanine aminotransferase; AMPK, adenosine monophosphate-activated protein kinase; AST, aspartate aminotransferase; BCAA, branched chain amino acid; Bcl-2, B-cell lymphoma 2; BMI, body mass index; CCl<sub>4</sub>, carbon tetrachloride; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CI, confidence interval; CLD, chronic liver disease; CTP, Child-Turcotte-Pugh; DAA, direct-acting antiviral; DDB, dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxypiphenyl-2,2'-dicarboxylate; FFA, free fatty acid; FLI, fatty liver index; FXR, farnesoid X receptor; GGT,  $\gamma$ -glutamyl transferase; GLUT, glucose transporter; GP1ba, glycoprotein 1ba; GSSG, glutathione disulfide; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HD-UDCA, high-dose UDCA; HE, hepatic encephalopathy; HMG-CoA, hydroxymethylglutaryl coenzyme A; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HR, hazard ratio; HSC, hepatic stellate cell; HVPG, hepatic venous pressure gradient; IFN, interferon; IL-6, interleukin-6; IRR, incident risk ratio; JAK, Janus kinase; LDL, low-density lipoprotein; LOLA, L-ornithine L-aspartate; LPS, lipopolysaccharides; LSEC, liver sinusoidal endothelial cell; MELD, Model for End-stage Liver Disease; mTOR, FULL NAME; NAD+, nicotinamide adenine dinucleotide+; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NF- $\kappa$ B, nuclear factor kappa-B; NKT, natural killer T; nor-UDCA, norursodeoxycholic acid; NRF2, nuclear factor erythroid 2-related factor 2; OR, odds ratio; OS, overall survival; PBC, primary biliary cholangitis; PI3K, phosphatidylinositol 3-kinase; PIVENS, Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis; PPAR, peroxisome proliferator-activated receptor; RCT, randomized controlled trial; ROS, reactive oxygen species; RR, risk ratio; STAT, signal transducer and activator of transcription protein; SVR, sustained virologic response; TGF, tumor growth factor; TLR, toll-like receptor; TNF, tumor necrosis factor; UDCA, ursodeoxycholic acid

ment but reduced by co-treatment of palmitate and UDCA.<sup>40</sup>

UDCA also protects hepatocytes from undergoing apoptosis.<sup>41</sup> There are multiple signaling pathways and mechanisms associated with the observed anti-apoptotic role of UDCA, such as the lowering of endoplasmic reticulum stress, enhancement of mitochondrial function and integrity, and ac-

centuation of survival signaling among the nuclear factor kappa-B (NF- $\kappa$ B), phosphatidylinositol 3-kinase (PI3K), and mitogen-activated protein kinase pathways.<sup>16</sup> Specifically, UDCA negatively regulates the mitochondrial apoptotic pathway by inhibiting Bax translocation and reinforcing B-cell lymphoma 2 (Bcl-2) activity (Fig. 1).<sup>42</sup> In stressed primary



**Figure 1.** Mechanisms for protective effects by UDCA, silymarin, DDB and its combination with other supplements, vitamin E, and aspirin in chronic liver diseases. UDCA and silymarin negatively regulate the mitochondrial apoptotic pathway by inhibiting Bax translocation, reinforcing Bcl-2 activity, and blocking the activations of caspase-3 and -12, which prevents the apoptosis of hepatocytes in chronic liver diseases. Moreover, UDCA, silymarin, vitamin E, and organosulfur (from garlic oil) relieve ROS-mediated oxidative stress in hepatocytes. Cytosolic FFA contributes to the intracellular ROS pool, and carnitine shuttles FFA-derived acyl-coenzyme A into the mitochondria, making them to undergo  $\beta$ -oxidation. TNF- $\alpha$ /IL-6 receptor signaling and TLR signaling upregulates the expression of pro-inflammatory cytokines via NF- $\kappa$ B activation, while UDCA, silymarin, DDB, vitamin E, and carnitine block this pathway in both hepatocytes and Kupffer cells. UDCA increases the secretion of bile acids via the upregulation of the hepatobiliary transporter genes, such *BSEP*, *MRP2*, and *MRP3*, and also enhances biliary bicarbonate excretion. Interestingly, UDCA induces neutral lipid accumulation in hepatocytes by exerting FXR-antagonistic effects. Aspirin attenuates intrahepatic inflammation by blocking platelet-derived, GP1ba-mediated Kupffer cell activation. UDCA, ursodeoxycholic acid; MRP, multidrug resistance protein; ROS, reactive oxygen species; Bcl-2, B-cell lymphoma 2; FFA, free fatty acid; FXR, farnesoid X receptor; TNF, tumor necrosis factor; IL-6, interleukin-6; DDB, dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxibiphenyl-2,2'-dicarboxylate; NF- $\kappa$ B, nuclear factor kappa-B; BSEP, bile salt export pump; GP1ba, glycoprotein 1ba; TLR, toll-like receptor.

rat hepatocytes, UDCA was shown to regulate the E2F-1/p53/Bax pathway to block apoptosis.<sup>42,43</sup> UDCA was also proven to target the miR-34a/SIRT1/p53 pro-apoptotic pathway in free fatty acid (FFA)-treated primary rat hepatocytes and the rat liver, reducing hepatocyte apoptosis.<sup>44</sup> Finally, these anti-apoptotic roles of UDCA block caspase-3 activation.<sup>42</sup>

Regarding steatosis in NAFLD livers, conflicting results have been offered. One recent report showed that UDCA induces neutral lipid accumulation in the liver in NAFLD patients by exerting farnesoid X receptor (FXR)-antagonistic effects.<sup>45</sup> On the contrary, another previous report showed that hepatic steatosis was decreased by UDCA in NAFLD rats, which was attributable to autophagy induction by activation of the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway.<sup>46</sup>

Importantly, UDCA also has immune-modulatory and anti-inflammatory effects in the liver. In patients with PBC, UDCA may alleviate auto-antigen-mediated liver injury by substantially reducing major histocompatibility complex class I expression in the liver.<sup>16,47</sup> In UDCA-fed, aged mice, inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ , C-C motif ligand 2, and interleukin-6 (IL-6) were significantly downregulated in liver and/or white adipose tissues relative to in the tissues of control mice.<sup>48</sup> These observed anti-inflammatory effects may occur as a consequence of the glucocorticoid receptor agonist activity of UDCA and resultant suppression of NF- $\kappa$ B-dependent inflammatory gene transcription in both parenchymal cells and non-parenchymal cells.<sup>49,50</sup> Moreover, UDCA impairs chemotaxis of liver-infiltrating T-cells by downregulation of intrahepatic interferon (IFN)- $\gamma$  and C-X3-C motif chemokine ligand 1 expression.<sup>51</sup> Interestingly, systemic administration of UDCA attenuates experimental auto-immune arthritis by suppressing T helper 17 cell differentiation through the upregulation of small heterodimer partner interacting leucine zipper protein and by inducing the activation of AMPK and p38 in mouse CD4 $^{+}$  T-cells.<sup>52</sup>

## Clinically beneficial effects

### NAFLD

In the past few decades, many clinical trials have been conducted to reveal whether UDCA has hepatoprotective effects in NAFLD as well as PBC. To date, however, such trials offer conflicting results because of different inclusion and exclusion criteria, treatment doses and durations, and combina-

tions with various drugs in each study (Table 1).

A non-randomized, 1-year prospective study in 40 patients showed that 13–15 mg/kg/day of UDCA produced significant improvements in liver enzymes and hepatic steatosis compared to clofibrate.<sup>53</sup> In a large randomized controlled trial (RCT) with 166 patients, Lindor et al.<sup>8</sup> showed that treatment with 13–15 mg/kg/day of UDCA over 2 years reduced alanine aminotransferase (ALT) levels to the same extent as placebo (mean change, -32.7 vs. -31.6 U/L;  $P=0.60$ ). However, there was no histologic benefit of UDCA treatment, although the proportion of subjects with improved steatosis was higher than that achieved with placebo, without statistical significance (46% vs. 37%;  $P=0.41$ ). High rates of ALT and steatosis improvement in the placebo group and a high dropout rate, particularly in the UDCA group (30%), might be issues warranting careful interpretation. In a subsequent randomized trial, Dufour et al.<sup>54</sup> did not show that UDCA had any significant benefit over placebo in promoting biochemical and histologic improvements either, although patients in the UDCA group experienced continuous decreases in aminotransferase levels over 2 years. Instead, UDCA and vitamin E combination therapy improved aminotransferase levels, liver histology, and metabolic profile in patients with nonalcoholic steatohepatitis (NASH).<sup>54,55</sup>

Based on the benefits of high-dose UDCA (HD-UDCA) in other CLDs,<sup>56,57</sup> two RCTs with HD-UDCA therapy were conducted. Leuschner et al. assigned 185 patients with biopsy-proven NASH to either HD-UDCA (23–28 mg/kg/day) or placebo treatment for 18 months.<sup>58</sup> HD-UDCA failed to improve overall histology over placebo, the primary endpoint, although lobular inflammation ( $P$  for NAFLD activity score [NAS]=0.005) and  $\gamma$ -glutamyl transferase (GGT) improved significantly (mean change, -52.53 vs. -16.84 U/L;  $P<0.0001$ ). On the other hand, Ratziu et al.<sup>59</sup> conducted another randomized trial in which a total of 126 patients with biopsy-proven NASH were randomized to receive HD-UDCA (28–35 mg/kg/day) or placebo for 1 year. The reductions in ALT level (-28.3% vs. -1.6% from baseline;  $P<0.001$ ) and FibroTest measure (median change, -10.5% vs. +9.6%;  $P<0.006$ ), which was used as a surrogate marker for fibrosis, was significantly greater in the HD-UDCA group than the placebo group. In addition, patients treated with HD-UDCA experienced significant reductions in serum glucose level and improved insulin resistance compared to patients in the placebo group. Different UDCA doses, treatment durations, and NAS at baseline

**Table 1.** Summary of clinical studies in UDCA

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Lindor et al. <sup>8</sup> (2004)	NAFLD	Biopsy-proven NASH with ALT >1.5×ULN	2 years	UDCA 13–15 mg/kg/day (80) Placebo (86)	45.4±12.0 48.5±11.6	· Changes in AST (mean, -21.7 U/L vs. -20.7 U/L; $P=0.37$ ) and ALT (mean, -32.7 U/L vs. -31.6 U/L; $P=0.60$ ) · Changes in steatosis (mean, -0.6 vs. -0.3; $P=0.41$ ), inflammation (mean, 0.0 vs. -0.1; $P=0.43$ ), and fibrosis (mean, 0.0 vs. 0.0; $P=0.50$ ) stages	RCT
Leuschner et al. <sup>58</sup> (2010)	NAFLD	Biopsy-proven NASH with ALT >1.5×ULN	18 months	UDCA 23–28 mg/kg/day (94) Placebo (91)	41.45 (18–71) 45.02 (18–73)	· Difference in modified Brunt score (mean, -0.98 vs. -0.97; $P=0.881$ ) · Difference in NAS (mean, -1.22 vs. -1.03; $P=0.355$ )	RCT
Ratziu et al. <sup>59</sup> (2011)	NAFLD	Biopsy-proven NASH with ALT >50 IU/L	12 months	UDCA 28–35 mg/kg/day (62) Placebo (61)	49.8±10.2 49.6±12.6	· Changes in ALT (-28.3% vs. -1.6%; $P<0.001$ ) · Proportion of ALT normalization (24.5% vs. 4.8%; $P<0.003$ ) · Changes in FibroTest values (median, -10.5% vs. +9.6%; $P<0.006$ )	RCT
Traussnigg et al. <sup>63</sup> (2019)	NAFLD	ALT >0.8×ULN	12 weeks	Nor-UDCA 1,500 mg/day (67) Nor-UDCA 500 mg/day (67) Placebo (64)	48.9±12.8 44.9±11.6 48.8±11.4	· Changes in ALT (mean, -17.2 U/L vs. -7.0 U/L vs. +5.3 U/L; $P<0.0001$ ) · Proportion of ALT < 0.8×ULN (17.5% vs. 14.8% vs. 5.2%) · Reduction of hepatic fat fraction by MRS (-23.5% vs. +0.9% vs. -1.0%)	RCT
Fabbri et al. <sup>64</sup> (2000)	CHC	Non-responders to IFN-α with ALT >1.5×ULN	14 months	IFN-α + UDCA 600 mg/day followed by UDCA 600 mg/day (53)	52.6±1.8	· Proportion of ALT normalization (38% vs. 12%; $P<0.01$ ) · Proportion of ALT relapse after withdrawal of IFN-α (55% vs. 100%; $P<0.01$ )	RCT
Boucher et al. <sup>65</sup> (2000)	CHC	Responders to IFN-α	12 months	UDCA 10 mg/kg/day (54) Placebo (53)	41±15 43±15	· Proportion of biochemical SR (30% vs. 46%; $P=NS$ ) · Proportion of virological SR (22% vs. 32%; $P=NS$ ) · Post-treatment Knodell score (mean, 6.6 vs. 5.6; $P=NS$ )	RCT
Onata et al. <sup>66</sup> (2007)	CHC	ALT >61 U/L	24 weeks	UDCA 150 mg/day (195) UDCA 600 mg/day (198) UDCA 900 mg/day (193)	58±12.2 57.7±12.0 59.8±10.1	· Changes in ALT (-15.3% vs. -29.2% vs. -36.2%; $P<0.001$ ) · Changes in AST (-13.6% vs. -25.0% vs. -29.8%; $P<0.001$ ) · Changes in GGT (-22.4% vs. -41.0% vs. -50.0%; $P<0.001$ )	RCT

Variables are expressed as median (interquartile range) or mean±standard deviation.  
UDCA, ursodeoxycholic acid; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ALT, alanine aminotransferase; ULN, upper limit of normal; AST, aspartate aminotransferase; RCT, randomized controlled trial; NAS, NAFLD activity score; nor-UDCA, norursodeoxycholic acid; MRS, magnetic resonance spectroscopy; CHC, chronic hepatitis C; IFN, interferon; SR, sustained response; NS, not significant.

might explain the discrepant results between the two studies.

Recently, norursodeoxycholic acid (nor-UDCA), a synthetic side chain-shortened homologue of UDCA,<sup>60</sup> was evaluated for the treatment of NAFLD based on promising results in pre-clinical studies.<sup>61,62</sup> In a randomized controlled, phase II clinical trial involving 198 patients, 1,500 mg/day of nor-UDCA significantly reduced serum ALT levels within 12 weeks (mean change, -17.2 vs. +5.3 U/L;  $P<0.0001$ ), and nor-UDCA was found to be safe and well tolerated.<sup>63</sup> In addition, hepatic fat fraction measured by magnetic resonance spectroscopy was remarkably reduced in nor-UDCA-treated patients (-23.5% vs. -1.0%).

Taken together, the results of various clinical trials suggest that conventional doses of UDCA (13–15 mg/kg/day) in monotherapy have little therapeutic effect in NASH, but HD-UDCA (28–35 mg/kg/day) may be beneficial in driving biochemical improvements in NASH patients with low severity. In addition, a conventional dose of UDCA may help to improve NASH when administered in combination with vitamin E. Further studies to evaluate the beneficial effects of nor-UDCA in patients with NAFLD or NASH are warranted.

### Viral hepatitis

The role of UDCA in viral hepatitis has not been studied extensively. There are a few studies evaluating the effect of UDCA in patients with chronic hepatitis C (CHC) with or without IFN treatment (Table 1). Fabbri et al.<sup>64</sup> conducted an RCT involving 103 patients who had not responded to IFN therapy. Patients were randomized to receive UDCA (600 mg/day) in addition to IFN or to continue on IFN- $\alpha$  alone. After stopping  $\alpha$ -IFN, patients who received UDCA continued to receive UDCA for an additional 6 months. UDCA improved the response rate to  $\alpha$ -IFN (ALT normalization, 38% vs. 12%;  $P<0.01$ ) and reduced the severity of relapse. In another RCT involving 107 biochemical responders to IFN, patients were randomized to receive either UDCA (10 mg/kg/day) or placebo for 12 months.<sup>65</sup> Continuation of UDCA therapy after withdrawal of IFN therapy did not significantly improve the maintenance of response to IFN or liver histology parameters in IFN responders. The most recent large-scale randomized trial was conducted in 596 CHC patients with detectable HCV RNA.<sup>66</sup> Patients were assigned randomly to receive 150, 600, or 900 mg/day of UDCA for 24 weeks, and it was found that 600 mg/day of UDCA was the optimal dose to decrease aspartate aminotransferase (AST) (-25.0% vs. -13.6% from baseline;

$P<0.001$ ) and ALT (-29.2% vs. -15.3% from baseline;  $P<0.001$ ) levels compared to 150 mg/day, while the GGT concentration was significantly lower in the 900-mg/day group than the 600-mg/day group (-50.5% vs. -41.0% from baseline;  $P<0.001$ ), which may indicate an improvement in cholestasis due to biliary injury in CHC. However, the serum HCV RNA did not change in any group.

Accordingly, UDCA may lead to an improvement in serum aminotransferase activities in CHC without effects on viral clearance. Since all studies were conducted in the IFN era, which carries a low treatment response rate, further considerations and studies are needed on the role of UDCA in CHC at this time of using DAAs.

### Safety

UDCA is widely used in the treatment of patients with PBC and has shown an excellent safety profile.<sup>67</sup> In viral hepatitis or NAFLD patients as well, no safety issues have been raised in various RCTs, even in studies with long-term administration of HD-UDCA. The most commonly reported adverse event of UDCA therapy is diarrhea,<sup>8,58,59,66</sup> although abdominal discomfort, fatigue, rash, and pruritus were also reported.<sup>8,58,59</sup> The rate of clinical adverse events was similar for UDCA and placebo when the conventional dose was administered, whereas diarrhea occurred more frequently with HD-UDCA than placebo.<sup>8,58,59</sup>

## SILYMARIN

### Mechanism of action

The bioactive extract of milk thistle, silymarin, has been documented to have several pharmacological features, including antioxidant and anti-inflammatory properties, in pre-clinical studies (Fig. 1).<sup>68-73</sup>

Silymarin uses scavengers, allowing for the elimination of free radicals; inhibits ROS producing-enzymes, preventing free radical formation; promotes protective molecule synthesis; and activates antioxidant enzymes.<sup>18</sup> In experimental studies, silybin, the most prevalent and biologically active flavonolignan isomer of silymarin, potently scavenges ROS such as hydroxyl and peroxy anions and hypochlorous acid.<sup>72,74-76</sup> Also, silybin inhibits superoxide anion radicals and nitric oxide in isolated Kupffer cells.<sup>68</sup> Silymarin enhances he-

patic glutathione generation by elevating cysteine availability and helps the liver to maintain glutathione by stabilizing membrane permeability through the inhibition of lipid peroxidation.<sup>69</sup> In an NAFLD mouse model, silymarin restored nicotinamide adenine dinucleotide+ (NAD+) homeostasis, sirtuin 1 activity, and the AMPK- $\alpha$  pathway to improve poly-(ADP-ribose)-polymerase function, which are important regulatory pathways linked to oxidative stress.<sup>77</sup> The antioxidant property of silymarin prevents or reduces hepatic inflammation by reducing oxidative stress in various liver diseases.<sup>78</sup>

Silymarin also exerts an anti-inflammatory effect. There is increasing evidence that silymarin inhibits inflammatory mediators, such as NF- $\kappa$ B, which is activated in most CLD, and inflammatory metabolites.<sup>79</sup> In isolated rat Kupffer cells, silymarin selectively inhibits leukotriene B4 formation, but weakly inhibited prostaglandin E2 formation, which may account for its anti-inflammatory action.<sup>68</sup> The anti-inflammatory property of silymarin may help to prevent or improve hepatic fibrogenesis given that chronic inflammation has been a common underlying mechanism in progressive liver fibrosis.<sup>80</sup>

Silymarin also has anti-fibrotic activity, inhibiting the conversion of hepatic stellate cells (HSCs) into myofibroblasts through the inhibition of fibrogenic pathways, including cytoskeletal formation, pro-fibrogenic collagen, and electron transfer chains. Animal and *in vitro* models demonstrate that silymarin down-regulates tumor growth factor (TGF)- $\beta$ 1 mRNA and inhibits NF- $\kappa$ B to improve hepatic fibrosis.<sup>79,81,82</sup> In an *in vitro* study, silybin inhibited the growth factor-induced production of pro-collagen in activated human HSC dose-dependently, slowing down the progression of early fibrosis.<sup>81</sup>

Insulin resistance is a well-known key mechanism in the pathogenesis of NAFLD. In a rat NAFLD model, silybin decreased insulin resistance by reducing visceral obesity, enhancing lipolysis, and inhibiting gluconeogenesis.<sup>83</sup> Silymarin can also restore a pathway of insulin receptor substrate-1/PI3K/Akt, which can reduce NAFLD-induced insulin resistance and steatosis, as well as activate the FXR, which in turn can decrease hepatic inflammation.<sup>26,84</sup>

## Clinically beneficial effects

### NAFLD

The clinical applications of silymarin in NAFLD have been

identified in several RCTs to date (Table 2).<sup>9,28,31,85-87</sup> Among them, two studies investigated the efficacy of silymarin in patients with histologically confirmed NAFLD.<sup>9,31</sup> In an RCT with 49 patients treated with 2,100 mg/day of silymarin and 50 patients treated with placebo, the proportion of patients who showed improvements in fibrosis ( $\geq 1$  stage) was significantly higher in the silymarin group compared to the placebo group (22.4% vs. 6.0%;  $P=0.023$ ), while the proportion of patients who had improvements in NAS ( $\geq 30\%$ ) was statistically comparable between the silymarin and placebo groups (32.7% vs. 26.0%;  $P=0.467$ ).<sup>31</sup> Another RCT also showed no statistically significant difference in the improvement of NAS ( $\geq 2$  points) between the silymarin and placebo groups (15–19% vs. 12%;  $P=0.79$ );<sup>9</sup> however, a retrospective cohort study demonstrated that patients with higher levels of oxidative stress markers had statistically significant improvements in NAS after silymarin treatment (variation, -70%;  $P=0.001$ ), while those with lower levels of oxidative stress markers did not (variation, -29%;  $P=0.057$ ), suggesting the effect of silymarin based on its antioxidant properties.<sup>88</sup> It is expected that further studies with selected subgroups of patients using relevant biomarkers representing the severity of oxidative stress or inflammation may reveal the clinically beneficial effect of silymarin in the histologic improvement of NAFLD more clearly.

The significant association between silymarin treatment and the improvement in aminotransferase levels in patients with NAFLD has been well reported in several RCTs.<sup>28,85-87</sup> Levels of AST (mean difference, -8.3 vs. -0.9 U/mL;  $P<0.001$ ) and ALT (mean difference, -9.3 vs. -0.6 U/mL;  $P<0.001$ ) significantly improved in the silymarin group compared to the placebo group.<sup>87</sup> In an RCT comparing pioglitazone, metformin, and silymarin, changes in AST (mean, -17.41 vs. -12.36 vs. -18.23;  $P=0.003$ ) and ALT (mean, -25.18 vs. -17.41 vs. -25.68;  $P<0.005$ ) levels after treatment were significantly different between the treatment groups.<sup>28</sup> Finally, a recent meta-analysis involving 622 patients with NAFLD revealed that silymarin was more efficacious than placebo in reducing ALT (mean difference, -14.86; 95% confidence interval [CI], -19.37 to -10.36;  $I^2=39\%$ ;  $P<0.001$ ) and AST (mean difference, -7.11; 95% CI, -14.16 to -0.05;  $I^2=88\%$ ;  $P<0.05$ ) levels.<sup>89</sup>

In addition, significant improvements in metabolic parameters, including levels of triglyceride, fasting glucose, and total cholesterol, as well as values of the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), waist circum-

**Table 2.** Summary of clinical studies in silymarin

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Navarro et al. <sup>9</sup> (2019)	NAFLD	NAS ≥4 without cirrhosis	48–50 weeks	Silymarin 1,260 mg/day (26) Silymarin 2,100 mg/day (27) Placebo (25)	47.3 (10.8) 48.2 (11.4) 49.5 (10.9)	· Improvement ≥2 in NAS (19% vs. 15% vs. 12%; $P=0.79$ ) · Normalized ALT level (8% vs. 25% vs. 5%; $P=0.08$ ) · Improved fibrosis stage (12% vs. 26% vs. 28%; $P=0.30$ )	RCT
Wha Kheong et al. <sup>31</sup> (2017)	NAFLD	NAS ≥4	48 weeks	Silymarin 2,100 mg/day (49) Placebo (50)	49.6±12.7 50.1±10.2	· Decrease ≥30% in NAS (32.7% vs. 26.0%; $P=0.467$ ) · Reductions in fibrosis ≥1 stage (22.4% vs. 6.0%; $P=0.023$ ) · Change in triglyceride level (mean, -0.2 vs. 0.04 mmol/L; $P=0.017$ )	RCT
Anushiravani et al. <sup>87</sup> (2019)	NAFLD	Grade ≥2 steatosis in ultrasonography	3 months	LSM (30) LSM + silymarin 140 mg/day (30)	47.0±9.1	· Changes in AST (mean, -0.9 vs. -8.3 U/ml; $P<0.001$ ) and ALT (mean, -0.6 vs. -9.3 U/ml; $P<0.001$ ) levels · Changes in WC (mean, -1.2 vs. -0.3 cm; $P<0.001$ )	RCT
Solhi et al. <sup>85</sup> (2014)	NAFLD	AST and ALT >1.2×ULN	8 weeks	LSM + silymarin 210 mg/day (33) LSM + placebo (31)	43.6±8.3 9.4±10.5	· Changes in AST (mean, 62.8 to 30.5 vs. 70.4 to 36.2; $P=0.038$ ) and ALT (mean, 91.3 to 38.4 vs. 84.6 to 52.3; $P=0.026$ ) levels (U/L)	RCT
Hajiaaghahmohammadi et al. <sup>28</sup> (2012)	NAFLD		8 weeks	Pioglitazone 15 mg/day (22) Metformin 500 mg/day (22) Silymarin 140 mg/day (22)	33.4±6.6 32.5±6.5 33.5±6.3	· Changes in AST (mean, 55.0 to 37.59 vs. 54.86 to 42.5 vs. 56 to 37.77; $P=0.03$ ) and ALT (mean, 77.45 to 52.27 vs. 78.36 to 60.95 vs. 78.73 to 53.05; $P=0.005$ ) levels (U/L) · Significant reductions in mean levels of fasting glucose, triglyceride, total cholesterol, and insulin and HOMA-IR in all groups ( $P<0.01$ )	RCT
Hashemi et al. <sup>86</sup> (2009)	NAFLD	AST and ALT >1.2×ULN or biopsy-proven NASH	6 months	Silymarin 280 mg/day (50) Placebo (50)	39.28±11.11 39.0±10.70	· ALT (52% vs. 18%; $P=0.001$ ) and AST (62% vs. 20%; $P=0.0001$ ) level normalization	RCT
Sorrentino et al. <sup>90</sup> (2015)	NAFLD	WC > 94 cm in men or >80 cm in women, triglyceride >150 mg/dL, and fasting glucose >100 mg/dL	90 days	LSM + silymarin 250 mg/day + vitamin E 60 IU/day (43) LSM (35)	56.63±12.79 55.40±13.63	· Change in hepatic steatosis index (mean, -1.85 vs. -0.19; $P=0.0134$ ) · Changes in BMI (mean, -0.71 vs. -0.004 kg/m <sup>2</sup> ; $P=0.022$ ) and WC (mean, -4.81 vs. -1.78 cm; $P=0.028$ )	Prospective cohort study

**Table 2.** Continued

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Stiuso et al. <sup>88</sup> (2014)	NAFLD	Biopsy-proven NASH	12 months	Low levels of TBARS: Silymarin 188 mg/day + phosphatidyl choline 388 mg/day + vitamin E acetate 50% 178.56 mg/day (11)	40.8±10.3	<ul style="list-style-type: none"> <li>· Proportion of change in NAS (-29%; NS), portal inflammation (-25%; NS), and fibrosis (-50%; <math>P=0.01</math>)</li> <li>· Proportion of change in AST (-33%; <math>P=0.05</math>), ALT (-14%; NS), and insulin (-8%; NS) levels and HOMA-IR (-11%; NS)</li> </ul>	Retrospective cohort study
				High levels of TBARS: silymarin 188 mg/day + phosphatidyl choline 388 mg/day + vitamin E acetate 50% 178.56 mg/day (19)		<ul style="list-style-type: none"> <li>· Proportion of change in NAS (-70%, <math>P=0.001</math>), portal inflammation (-58%; <math>P=0.001</math>), and fibrosis (-60%; <math>P=0.001</math>)</li> <li>· Proportion of change in AST (-42%; <math>P=0.01</math>), ALT (-31%; <math>P=0.05</math>), and insulin (-40%; <math>P=0.001</math>) levels and HOMA-IR (-42%; <math>P=0.001</math>)</li> </ul>	
Fried et al. <sup>91</sup> (2012)	HCV	ALT ≥65 U/L who were unsuccessfully treated with IFN	24 weeks	Silymarin 1,260 mg/day (50) Silymarin 2,100 mg/day (52) Placebo (52)	54.0 (52.0–57.0) 54.0 (48.0–58.0) 56.0 (51.5–59.5)	<ul style="list-style-type: none"> <li>· Proportion of ALT ≤45 U/L (4.0% vs. 3.8% vs. 1.9%; <math>P=0.80</math>), at least 50% ALT decline and ALT &lt;65 U/L at week 24 (2.0% vs. 3.8% vs. 3.8%; <math>P=0.83</math>)</li> <li>· Changes in ALT (mean, -14.4 vs. -11.3 vs. -4.3 U/L; <math>P=0.75</math>) and HCV RNA (mean, -0.03 vs. 0.04 vs. 0.07 log<sub>10</sub> IU/L; <math>P=0.54</math>) levels</li> </ul>	RCT
Yakoot and Salem <sup>92</sup> (2012)	HCV	Genotype 4, IFN-naïve or relapsers/non-responders to IFN or combined therapy	6 months	Silymarin 420 mg/day (29) Spirulina 1,500 mg/day (30)	48±12 47±12	<ul style="list-style-type: none"> <li>· ETR (3.4% vs. 13.3%; <math>P=0.12</math>)</li> <li>· 3 months virological response (0% vs. 3.3%; <math>P=0.22</math>)</li> <li>· Reduction in ALT level (mean, -6.8 vs. -23.7 IU/L; <math>P=0.006</math>)</li> </ul>	RCT
Tanamly et al. <sup>94</sup> (2004)	HCV	Presence of HCV RNA	12 months	Silymarin 373.5 mg/day (68) Multivitamin (71)	44.1	<ul style="list-style-type: none"> <li>· All physical and mental health variables (SF-36 questionnaire) were improved in both groups.</li> <li>· Persistent HCV RNA (97.1% vs. 95.8%; <math>P=0.684</math>) and ALT &gt;35 IU/L (13.0% vs. 14.3%; NS)</li> </ul>	RCT
Ferenci et al. <sup>95</sup> (2008)	HCV	Nonresponders to full-dose PegIFN/RBV	14 days	Silymarin iv 5 (3), 10 (3), 15 (5), 20 (9) mg/kg + 24 weeks PegIFNa-2a 180 µg/week + ribavirin 1–1.2 g/day from day 8	52.7±12.8	<ul style="list-style-type: none"> <li>· Dose-dependent HCV RNA decrease (log drop after 14 days: mean, 1.63 [5 mg/kg, 4.16] [10 mg/kg], 3.69 [15 mg/kg], and 4.85 [20 mg/kg]; <math>P&lt;0.001</math>)</li> <li>· Undetectable HCV RNA in 7 patients on 15 or 20 mg/kg of silymarin at week 12</li> </ul>	Prospective cohort study

Variables are expressed as median (interquartile range) or mean±standard deviation.

NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; ALT, alanine aminotransferase; RCT, randomized controlled trial; LSM, lifestyle modification; AST, aspartate aminotransferase; WC, waist circumference; ULN, upper limit of normal; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; NASH, nonalcoholic steatohepatitis; BMI, body mass index; TBARS, thiobarbituric acid-reactive species; NS, not significant; HCV, hepatitis C virus; IFN, interferon; ETR, end-treatment response; PegIFN/RBV, pegylated interferon and ribavirin.

ference, and body mass index (BMI) following treatment with silymarin have been reported in previous RCTs and a prospective cohort study, suggesting the potential of silymarin for the treatment of metabolic syndrome.<sup>28,31,87,90</sup>

Overall, silymarin treatment in NAFLD seems beneficial in improving hepatic necro-inflammation, as reflected by the improvement of AST and ALT levels, and metabolic parameters. Therefore, silymarin may be a potential future therapy for patients with NASH, presumably in combination with other agents, but still requires large RCTs for solid validation.

### Viral hepatitis

The beneficial effect of silymarin in improving aminotransferase in CHC patients remains controversial despite several RCTs and prospective studies (Table 2).<sup>91-95</sup> In an RCT with CHC patients in whom IFN therapy was unsuccessful, the proportion of patients who had ALT normalization was statistically comparable between the silymarin and placebo groups (3.8–4.0% vs. 1.9%;  $P=0.80$ ).<sup>91</sup> Another RCT with CHC patients who were treatment-naïve or relapsers/non-responders to IFN or combined therapy showed a significant improvement in ALT level in a spirulina group compared to the silymarin group (mean, -23.7 vs. -6.8 IU/L;  $P=0.006$ ).<sup>92</sup> In contrast, a prospective study reported significant reductions in levels of ALT (mean, 108.7–70.3 IU/L;  $P<0.001$ ) and AST (mean, 99.4–59.7 IU/L;  $P=0.004$ ) after silymarin treatment.<sup>93</sup> Two prospective studies demonstrated significant reductions in HCV RNA levels after silymarin treatment; however, silymarin had no effect in the suppression of HCV RNA in all RCTs.<sup>91-95</sup>

### Safety

In clinical trials, silymarin has been used for up to 48 weeks at 2,100 mg/day.<sup>9,31</sup> Overall, silymarin is well tolerated in CLD patients with no or only a very low incidence of serious adverse events.<sup>9,28,31,85-88,90-95</sup> Systematic reviews demonstrated a 4% incidence of adverse events and no treatment-related serious adverse events or deaths.<sup>89,96</sup>

## DIMETHYL-4,4'-DIMETHOXY-5,6,5',6'-DIMETHYLENEDIOXBIPHENYL-2,2'-DICARBOXYLATE (DDB) AND ITS COMBINATION WITH OTHER SUPPLEMENTS

### Mechanism of action

DDB is a synthetic compound derived from schisandrin C, an active metabolite from *Schizandrae sinensis* Fructus. It has been widely used in practice to lower ALT levels in chronic hepatitis for nearly 50 years.<sup>97</sup> The protective roles of DDB were reported in experimental models of liver injury using carbon tetrachloride ( $CCl_4$ ), D-galactosamine, thioacetamide, and prednisolone.<sup>19</sup> It is known to reduce the membranal lipid peroxidation and ALT release from damaged hepatocytes (Fig. 1).<sup>19,98</sup> DDB has also shown additional inhibitory effects on lipopolysaccharides (LPS)-inducible NF- $\kappa$ B activation and subsequent TNF- $\alpha$  production.<sup>99</sup> It is related to the inhibition of either I $\kappa$ B $\alpha$  degradation or signaling of caspases-3, -8, and -9.<sup>99,100</sup>

Carnitine orotate complex or diallyl sulfide from garlic oil has been used in combination with DDB to add clinical benefit with different mechanisms of action. A DDB-carnitine orotate complex prevented FFA-induced lipotoxicity by adding carnitine (Fig. 1). In a physiologic state, carnitine shuttles long-chain fatty acids into mitochondria and facilitates mitochondrial  $\beta$ -oxidation by acting as a coenzyme for palmitoyl-transferase 1A.<sup>101</sup> In an *in vitro* study, carnitine facilitated effective mitochondrial  $\beta$ -oxidation, thereby reducing both intracellular fat deposits and alternative fat peroxidation, which ultimately leads to decreased production of ROS and oxidative stress.<sup>102</sup> Carnitine treatment in diabetic rats fed a methionine choline-deficient diet led to decreased serum ALT levels and improved lobular inflammation *in vivo*.<sup>102</sup> Meanwhile, diallyl sulfide, one of the organosulfur compounds from garlic oil, is known to inhibit the action of CYP450 2E1.<sup>103,104</sup> It modulates the production of toxic or reactive intermediate during phase II detoxification (Fig. 1).<sup>105</sup> Garlic oil enhanced the protective effect of DDB in the improvement of serum ALT level and was linked to decreasing numbers of Kupffer cells and dead hepatocytes in  $CCl_4$ -treated rats.<sup>106</sup> Meanwhile, in case of alcohol-induced hepatotoxicity, it was only blocked by adding garlic oil to DDB (~40%) and not by DDB only. Furthermore, Park et al.<sup>107</sup> reported that garlic oil combined with DDB was protective of glutathione

deficiency-induced liver injury as evidenced by improved ALT and triglyceride levels. Garlic oil combined with DDB showed a synergistic benefit according to a comparison of its histologic activity to that of treatment with only DDB or garlic oil.

## Clinically beneficial effects

### CLD

Apart from the widespread experimental studies with long histories, well-designed, RCTs are limited, involving only a small number of participants and short duration of treatment (Table 3). In the design of a double-blind, active-controlled trial, participants with CLDs (NASH, 69%; alcoholic hepatitis, 20%; chronic hepatitis, 11%) were treated with either DDB (750 mg/day) or UDCA (300 mg/day) for 24 weeks,<sup>10</sup> and DDB led to a significantly higher rate of ALT normalization compared to UDCA (80.0% vs. 34.8%,  $P<0.001$ ).

Supplementing DDB with carnitine orotate complex was evaluated in two RCTs. In a phase II RCT for CLDs other than viral hepatitis, 8 weeks of supplementing DDB (150 mg) with carnitine orotate complex (900 mg) led to a significantly higher rate of ALT normalization (88.5%) compared to combined carnitine orotate complex (600 mg) and DDB (150 mg) (54.5%) treatment or treatment with DDB (150 mg) only (44.4%) ( $P=0.003$ ).<sup>108</sup> In a phase III trial of participants with CLDs other than viral hepatitis,<sup>109</sup> adding a high dose of carnitine orotate complex (900 mg) to DDB (150 mg) led to a higher rate of ALT normalization (81.1%) than adding a low dose of carnitine orotate complex (600 mg) to DDB (100 mg) (67.4%) or treating with DDB (100 mg) alone (64.5%) ( $P=0.041$ ) (Table 3).

The addition of garlic oil to DDB was recently evaluated in a double-blind RCT with 12 weeks of intervention (Table 3).<sup>110</sup> Kim et al.<sup>110</sup> evaluated the beneficial effect on ALT normalization, improvement of quality of life, and safety of the DDB-garlic oil complex in patients with CLDs other than viral hepatitis. The rate of ALT normalization was 89% in the DDB-garlic oil complex group, which was significantly higher than that of the silymarin group (18.6%) or placebo group (22.9%) ( $P<0.001$ ). The level of serum malondialdehyde, a lipid peroxidation marker, was decreased in the DDB-garlic oil complex group (-1.4 pmol/mg), but increased in both the silymarin-control and placebo groups ( $P<0.001$ ). Although the score for total CLD questionnaire score was significantly improved within the DDB-garlic oil complex group after treatment, the

score improvement was statistically similar among the three treatment groups.

In summary, DDB and its combination with other supplements seems to significantly decrease the serum level of ALT in patients with CLDs other than viral hepatitis, when compared to silymarin or placebo.

### NAFLD

Given the benefit of carnitine on the reduction in intracellular FFA level and the improvement of insulin resistance, the effect of a DDB-carnitine orotate complex was evaluated in participants with either impaired fasting glucose metabolism or type 2 diabetes (Table 3). In a double-blind RCT, either metformin and placebo or metformin and a DDB-carnitine orotate complex was given for 12 weeks to participants with both impaired fasting glucose metabolism and NAFLD.<sup>112</sup> Even with the small number of patients in each group, the reduction in ALT from baseline was significantly greater in the metformin and DDB-carnitine orotate complex group (mean reduction,  $51.5\pm33.2$  IU/L) than in the metformin and placebo group (mean reduction,  $16.7\pm31.3$  IU/L) among the patients with impaired fasting glucose metabolism and NAFLD ( $P=0.001$ ). The change in 8-hydroxy-2'-deoxyguanosine, an oxidative stress marker, was  $0.7\pm3.2$   $\mu$ g/g in the metformin and DDB-carnitine orotate complex group and  $-1.2\pm2.9$   $\mu$ g/g in the metformin and placebo group ( $P=0.034$ ), suggesting a benefit of the DDB-carnitine orotate complex in decreasing oxidative stress. Furthermore, the fold change ( $2^{-\Delta\Delta Ct}$ ) in mitochondrial copy number was significantly greater in the metformin and DDB-carnitine orotate complex group than that of the metformin and placebo group ( $1.16\pm0.38$  vs.  $0.95\pm0.45$ ,  $P<0.05$ ), suggesting the occurrence of less mitochondrial damage in the DDB-carnitine orotate complex group. Nevertheless, the additional effects of the DDB-carnitine orotate complex on the changes of fasting plasma glucose, C-peptide, insulin, HbA1c, and HOMA-IR were not significant.

In another multicenter, double-blind, placebo-controlled RCT, Bae et al.<sup>112</sup> evaluated the effect of adding a DDB-carnitine orotate complex to anti-diabetic treatment on ALT normalization in patients with type 2 diabetes and NAFLD. At 12 weeks of treatment, the rate of ALT normalization was significantly higher in the DDB-carnitine orotate complex treatment group than the placebo group (89.7% vs. 17.9%,  $P<0.001$ ). The liver attenuation index according to non-con-

**Table 3.** Summary of clinical studies in DDB

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Lee et al. <sup>108</sup> (2014)	CLD	(1) Persistent ALT $\geq 1.5 \times ULN$ more than once during the previous 6 months; (2) ALT $\geq 1.5 \times ULN$ at enrollment	24 weeks	DDB 750 mg/day (67)	51 (20–72)	ALT normalization ( $\leq 40$ IU/L) (80.0% vs. 34.8%; $P < 0.001$ )	RCT; double blind, active-controlled
Kang et al. <sup>108</sup> (2001)	CLD	(1) Biopsy-proven chronic hepatitis or (2) abnormal AST/ALT for >6 months; no evidence of viral hepatitis B or C	8 weeks	High-dose group: DDB 150 mg + carnitine/orotate complex 900 mg/day (33) Low-dose group: DDB 150 mg + carnitine/orotate complex 600 mg/day (30)	49.03 $\pm$ 9.74 41.58 $\pm$ 11.73	ALT normalization (88.5% vs. 54.6% vs. 44.4%; $P = 0.0027$ )	RCT; double blind, phase II
Park et al. <sup>109</sup> (2001)	CLD	(1) Biopsy-proven chronic hepatitis or (2) ALT elevation ( $\geq 1.5 \times ULN$ ) more than twice during the previous 6 months; no evidence of viral hepatitis B or C	8 weeks	High-dose group: DDB 150 mg + carnitine/orotate complex 900 mg/day (53) Low-dose group: DDB 100 mg + carnitine/orotate complex 600 mg/day (48)	44.57 $\pm$ 11.49 43.24 $\pm$ 13.01	ALT normalization (81.13% vs. 67.35% vs. 64.54%; $P = 0.0407$ )	RCT; double blind, phase III
Kim et al. <sup>110</sup> (2014)	CLD	(1) Abnormal ALT or AST in previous 6 months, (2) sonographical findings of chronic hepatitis or fatty liver, or (3) history of being treated for chronic hepatitis for >30 days	12 weeks	DDB + garlic oil 960 mg/day (100) Silymarin 1,018 mg/day (102) Placebo (35)	44 (20–79) 49 (20–75) 44 (24–77)	ALT normalization ( $\leq 40$ IU/L) (89% vs. 18.6% vs. 22.9%; $P < 0.001$ )	RCT; double blind, placebo- and active-controlled, phase IV

**Table 3.** Continued

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Hong et al. <sup>111</sup> (2014)	NAFLD	Combined with impaired fasting glucose metabolism	12 weeks	Metformin 750 mg/day and DDB-carnitine orotate complex 900 mg/day (26) Metformin 750 mg/day and placebo (26)	51.5±9.4 52.0±9.6	Decrement of ALT level (mean, 51.5 vs. 16.7; $P=0.001$ )	RCT; double blind, placebo-controlled
Bae et al. <sup>112</sup> (2015)	NAFLD	Combined with type 2 diabetes	12 weeks	DDB-carnitine orotate complex 824 mg/day (39) Placebo (39)	50.6±9.3 52.0±9.4	ALT normalization (<30 IU/L in men or <19 IU/L in women) (89.7% vs. 17.9%; $P<0.001$ )	RCT; double blind, placebo-controlled
Jun et al. <sup>113</sup> (2013)	HBV	(1) ALT ≥80 IU/L, (2) ALT < ULN×10, (3) treatment-naïve, and (4) HBV >105 copies/ml in case of HBeAg-positive result or HBV >104 copies/ml in case of HBeAg-negative result	12 months	Entecavir 0.5 mg/day and DDB-carnitine orotate 2,472 mg/day complex (67) Entecavir 0.5 mg/day (63)	43.0 ± 9.8 44.9±10.0	ALT normalization (<40 IU/L) (100% vs. 85.7%; $P=0.0019$ )	RCT

Variables are expressed as median (interquartile range) or mean±standard deviation.  
DDB, dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxypyrene-2,2'-dicarboxylate; CLD, chronic liver disease; ALT, alanine aminotransferase; ULN, upper limit of normal; RCT, randomized controlled trial; AST, aspartate aminotransferase; NAFLD, nonalcoholic fatty liver disease; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen.

trast computed tomography was also significantly increased in the DDB-carnitine orotate complex group compared to the placebo group ( $6.21\pm8.96$  vs.  $0.74\pm8.05$  Hounsfield units,  $P=0.008$ ), indicating that the DDB-carnitine orotate complex has a beneficial effect in terms of the improvement of steatosis among patients with both type 2 diabetes and NAFLD.

In summary, a DDB-carnitine orotate complex is effective in ALT normalization in NAFLD, but its beneficial effect on the improvement of insulin resistance in NAFLD combined with either impaired fasting glucose metabolism or type 2 diabetes should be further investigated to gather solid evidence. Additionally, there is an unmet need for further data supporting the effects of DDB-containing drugs on the improvement of liver histology.

### Viral hepatitis

The beneficial effect of a DDB-carnitine orotate complex in ALT normalization was evaluated in treatment-naïve chronic hepatitis B (CHB) patients with concomitant use of entecavir (Table 3).<sup>113</sup> Despite there being no effect on virologic response, the DDB-carnitine orotate complex combined with entecavir led to a higher rate of ALT normalization (100%) compared to entecavir treatment only (85.7%) after 12 months ( $P=0.002$ ). This might indicate that a DDB-carnitine orotate complex can synergistically stabilize hepatic necroinflammation during antiviral therapy for CHB.

### Safety

Except for a mild degree of skin urticaria,<sup>10</sup> no severe adverse events related to DDB have been reported. Adverse events supposed to have a causal relationship with DDB and carnitine orotate complex use include abdominal discomfort, indigestion, headache, and nausea.<sup>109,113</sup> Rates of adverse events did not differ among the control, low-dose, and high-dose groups using DDB and carnitine orotate complex.<sup>109</sup> The adverse events possibly related to DDB-garlic oil treatment were diarrhea, dry mouth, epigastric soreness, rash, and so on.<sup>111</sup> The rates of adverse events were not different compared to those of the placebo control group or active control (silymarin) group, and no serious adverse events occurred during the 12 weeks of the study period.<sup>110</sup>

## L-ORNITHINE L-ASPARTATE (LOLA)

### Mechanism of action

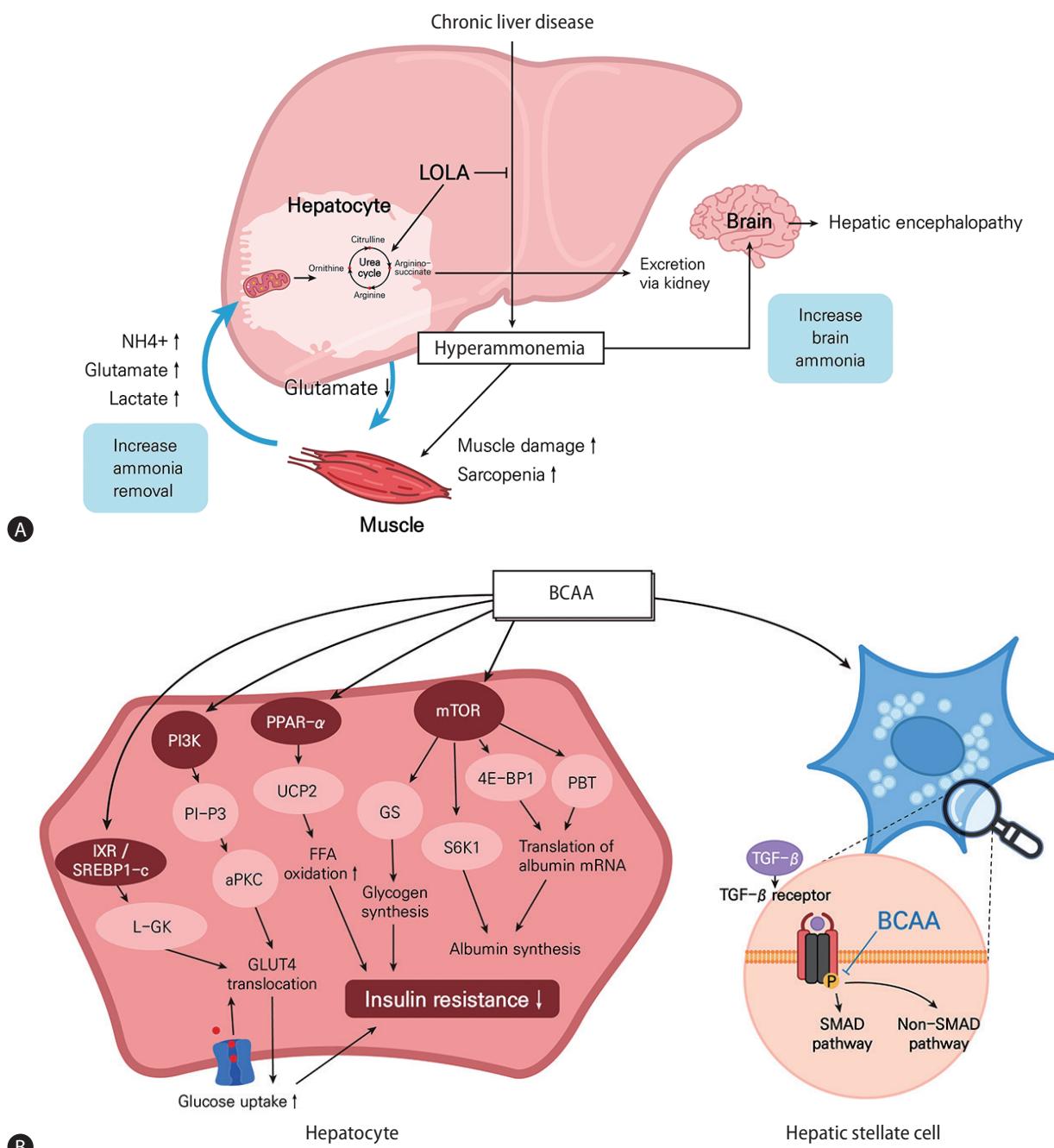
LOLA is a mixture of endogenous amino acids that promotes ammonia removal in patients with liver cirrhosis.<sup>114,115</sup> Ammonia is produced as a result of nitrogen metabolism in muscle and other peripheral tissues.<sup>114</sup> In the liver, ammonia is converted into urea, which is excreted in urine.<sup>116</sup> Impaired liver function can result in an elevated ammonia concentration.<sup>117</sup> In experimental models, the expression levels of genes encoding urea-cycle enzymes, as well as the amounts of those enzymes, are reduced, suppressing ureagenesis and inducing hyperammonemia in a pre-cirrhotic state.<sup>21</sup> Hyperammonemia may trigger fibrosis progression in patients with NASH.

LOLA promotes ammonia removal by increasing the synthesis of urea (Fig. 2A). Hypermethylation of urea cycle-related genes and reduced quantities and activities of urea-cycle enzymes have been noted in patients with NASH.<sup>20</sup> These changes increase the plasma ammonia concentration and result in ammonia accumulation in the liver tissue of patients with NASH. LOLA reduces the serum concentrations of liver enzymes and triglycerides in patients with NASH. The underlying mechanisms of this effect may be enhanced by ammonia removal, increased antioxidant activity, attenuated lipid peroxidation by glutamine and GHS, and improved hepatic microcirculation by L-arginine-derived nitric oxide.

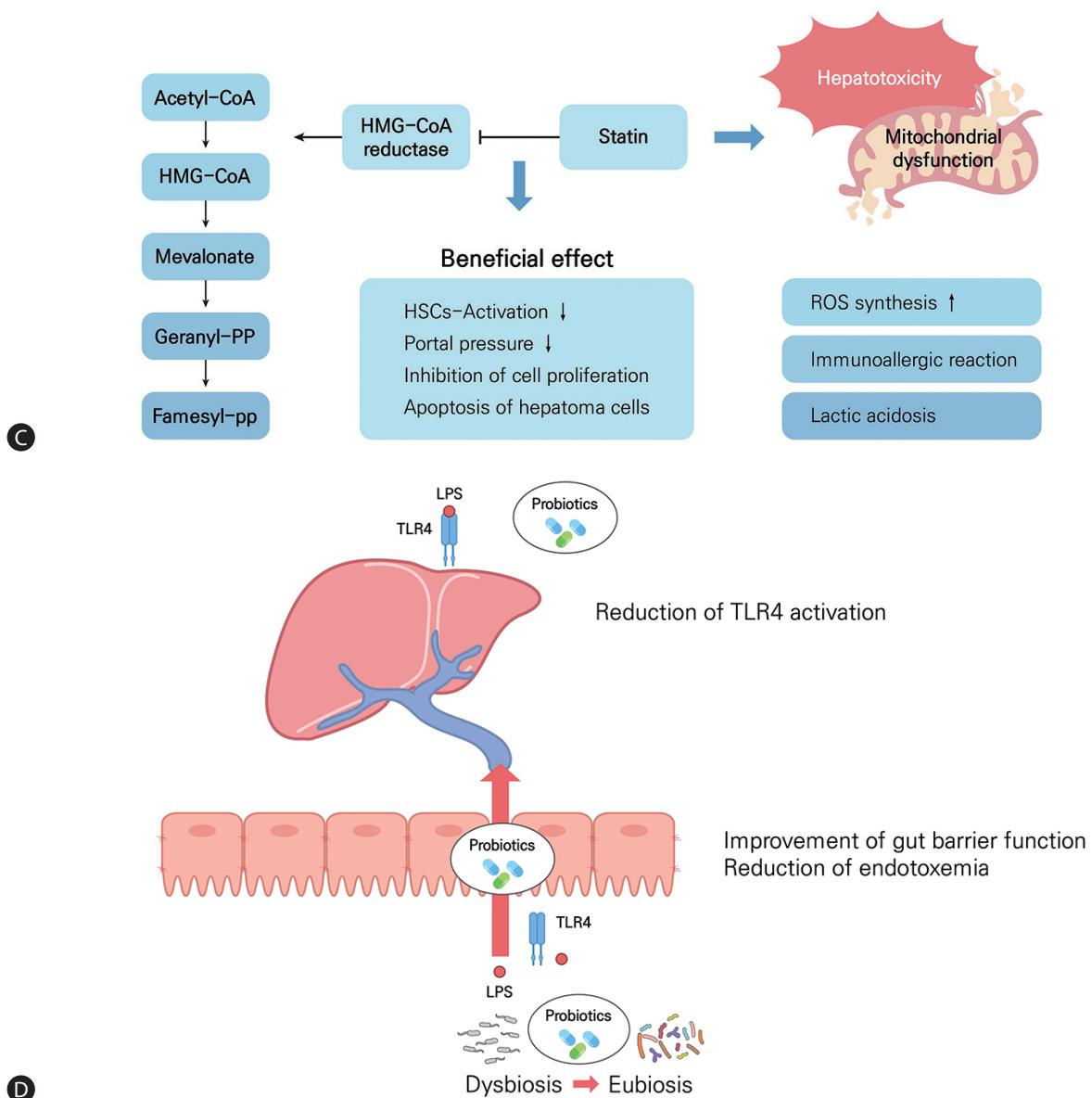
### Clinically beneficial effects

#### NAFLD

Few studies have evaluated LOLA as a treatment for NASH. A total of 463 patients with fatty liver, 29% of whom had NAFLD, were treated with LOLA during 1–3 months.<sup>96</sup> It was determined that LOLA reduced serum AST, ALT, and GGT levels by up to 70% in patients with CLD (AST, from mean of  $48.1\pm53.7$  to  $25.7\pm16.1$  U/L; ALT, from mean of  $52.6\pm44.7$  to  $39.2\pm36.5$  U/L; GGT, from mean of  $155.4\pm236.7$  to  $60.9\pm56.3$  U/L).<sup>96</sup> Moreover, beneficial treatment outcomes were more pronounced in patients with fatty liver than those with cirrhosis.<sup>118</sup> In a multicenter open-label, multidose RCT,<sup>119</sup> the efficacy of LOLA was assessed in 72 patients with NASH. Patients were prescribed high-dose (6 g bid; n=38) or low-dose (3 g bid; n=34) LOLA for 12 weeks. After 6 and 12 weeks of



**Figure 2.** Mechanisms and effects of LOLA, BCAA, statins, and probiotics in chronic liver diseases. (A) In patients with chronic liver disease, hepatic ammonia removal is decreased and muscle ammonia removal is increased. LOLA acts to prevent hyperammonemias by increasing the synthesis of urea. (B) BCAA treatment acts on hepatocytes to decrease insulin resistance and affects albumin synthesis and acts on stellate cells to inhibit fibrosis by regulating TGF- $\beta$  pathways. (C) Statins inhibit HMG-CoA reductase and induce pleiotropic effects by the deactivation of hepatic stellate cells, reduction of portal pressure, and inhibition of cell proliferation and induction of hepatoma cells. The liver toxicity of statins can be mediated by mitochondrial dysfunction, ROS synthesis, immuno-allergic reactions, and lactic acidosis. (D) Therapeutic effects of probiotics modulating the gut microbiota and the gut-liver axis to improve liver diseases. LOLA, L-ornithine L-aspartate; BCAA, branched chain amino acid; PI3K, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferator-activated receptor; mTOR, mammalian target of rapamycin; L-GK, liver type glucokinase; UCP2, uncoupling protein 2; GS, glycogen synthase; 4E-BP1, 4E-binding protein 1; GLUT, glucose transporter; TGF, tumor growth factor; BCAA, branched chain amino acid; SMAD, suppressor of mothers against decapentaplegic; HMG-CoA, hydroxymethylglutaryl coenzyme A; HSC, hepatic stellate cell; ROS, reactive oxygen species; LPS, lipopolysaccharides; TLR, toll-like receptor.



**Figure 2.** Continued.

treatment, the serum levels of AST (baseline: mean,  $82.28 \pm 29.92$ ; 6 weeks: mean,  $66.64 \pm 29.17$ ; 12 weeks: mean,  $61.86 \pm 26.69$  U/L), ALT (baseline: mean,  $106.95 \pm 39.90$ ; 6 weeks: mean,  $84.10 \pm 38.98$ ; 12 weeks: mean,  $65.80 \pm 26.73$  U/L), and GGT (baseline: mean,  $114.29 \pm 44.72$ ; 6 weeks: mean,  $90.10 \pm 34.96$ ; 12 weeks: mean,  $70.87 \pm 23.57$  U/L) were significantly lowered in both groups than at baseline. Additionally, LOLA resulted in a significant dose-related reduction in the levels of ALT (low-dose vs. high dose group: 6-week mean,  $22.85 \pm 26.88$  vs.  $35.92 \pm 32.28$  U/L;  $P < 0.0001$ ; 12-week mean,

$41.15 \pm 24.07$  vs.  $50.19 \pm 28.08$  U/L;  $P < 0.0001$ ).<sup>119</sup> In another study with 78 patients with NASH, LOLA improved hepatic microcirculation as evaluated by polyhepatography (a modified technique for the non-invasive estimation of intrahepatic blood flow) in the presence of stage 0–1 fibrosis.<sup>120</sup> However, the gathered data were limited due to the small sample size and use of serum transaminase as the main outcome. Thus, further studies of the effect of LOLA in patients with NAFLD are needed.

### Hepatic encephalopathy (HE)

HE is a severe neuropsychiatric complication of cirrhosis,<sup>121</sup> characterized by deficits in attention, visuospatial construction, and impaired motor speed. Hyperammonemia is consistently reported in such patients. Treatment strategies principally seek to lower the levels of circulating ammonia. In a systematic review and meta-analysis, LOLA appeared to improve the mental state (pooled risk ratio [RR], 1.36; 95% CI, 1.10–1.69;  $P=0.005$ ) and lower the ammonia level (mean difference, -17.50  $\mu\text{mol/L}$ ;  $P=0.0008$ ) of patients with overt HE or minimal HE.<sup>115</sup> A recent double-blind, randomized, placebo-controlled trial showed that combining intravenous LOLA with lactulose and rifaximin significantly improved the HE grade (92.5% vs. 66%;  $P<0.001$ ), shortened the recovery time (mean,  $2.70\pm0.46$  vs.  $3.00\pm0.87$  days;  $P=0.03$ ), and reduced the 28-day mortality rate (16.4% vs. 41.8%;  $P=0.001$ ) compared to only lactulose and rifaximin use.<sup>24</sup> In addition, the LOLA group showed significantly higher reductions in levels of blood ammonia (mean,  $51.69\pm10.835$  vs.  $37.52\pm12.41$   $\mu\text{mol/L}$ ;  $P<0.001$ ) and inflammatory markers such as IL-6 (mean,  $36.43\pm27.51$  vs.  $26.93\pm20.55$  pg/mL;  $P=0.025$ ) and TNF- $\alpha$  (mean,  $10.83\pm5.12$  vs.  $8.77\pm5.56$ ;  $P=0.027$ ), compared to the placebo group.<sup>24</sup>

### Safety

The rate of adverse events was low (4.4–4.8%) and most were gastrointestinal-related.<sup>119</sup>

## BRANCHED CHAIN AMINO ACIDS (BCAAs)

### Mechanism of action

BCAAs are some of the essential amino acids and consist of leucine, valine, and isoleucine. In basic research, BCAAs have been studied in relation to metabolism, liver fibrosis, and immunity (Fig. 2B). First, with respect to metabolism, BCAAs were reported to have major effects on 1) protein/albumin synthesis and 2) glucose/lipid metabolism and insulin resistance. Among the BCAAs, leucine, in particular, increases albumin production.<sup>22</sup> Specifically, leucine activates the mTOR pathway and increases the transcription of albumin mRNA by increasing 4E-binding protein downstream of the mTOR pathway.<sup>122–124</sup> In addition, in HepG2 cells, a mechanism by which BCAA-stimulated polypyrimidine tract-binding protein

binds to albumin mRNA and increases albumin translation has been reported.<sup>125</sup> The mechanisms by which BCAAs affect glucose metabolism have also been variously reported, such as the PI3KAkt pathway,<sup>126,127</sup> induced glucose transporter (GLUT)-4 and GLUT-1 translocation, and increased glycogen synthase activity.<sup>128</sup> BCAAs improve insulin resistance through liver, skeletal muscle, and adipose tissue, respectively, and PI3K, peroxisome proliferator-activated receptor (PPAR)- $\alpha$ , and Akt pathways are involved.<sup>126,128–130</sup> Recently, it has been reported that BCAA administration inhibits the lipogenesis-related genes *FAS* and *ACC* through the proliferation of intestinal flora, thereby inhibiting lipogenesis.<sup>131</sup> Also, BCAA administration can help improve hepatic fibrosis, and the main mechanism is inhibition of the TGF- $\beta$  signaling pathway of HSCs.<sup>132–135</sup> Lastly, among BCAAs, valine especially can help to restore immune function by regulating the maturation and function of monocyte-derived dendritic cells in cirrhotic patients.<sup>136</sup>

### Clinically beneficial effects

#### Viral hepatitis

In viral hepatitis, BCAA administration improved HOMA-IR in insulin-resistant CHC patients (mean after treatment, 4.5 in the BCAA group vs. 5.3 in the control group;  $P=0.047$ ).<sup>29</sup> Also, 2-year BCAA administration in obese CHC patients was effective in preventing HCC and improving IFN signaling promoted by malnutrition (event-free survival for HCC: hazard ratio [HR], 0.3; 95% CI, 0.12–0.78,  $P=0.008$ ) (Table 4).<sup>137</sup>

#### Liver cirrhosis

In patients with liver cirrhosis, the effect of BCAA on the prognosis of cirrhotic patients has been verified through various RCTs (Table 4). BCAA administration for >6–12 months has been commonly reported to reduce clinical decompensation (14.8% vs. 30.4%;  $P=0.043$ ).<sup>138</sup> In particular, BCAA was effective in reducing the occurrence of varix rupture, the rate of hepatic failure, and incidence of de novo HE.<sup>138–143</sup> BCAA was also found to be helpful for improving aspects of the quality of life (i.e., physical functioning improved from  $67\%\pm4\%$  to  $73\%\pm3\%$ ;  $P=0.023$ ).<sup>144</sup> such as sleep disturbance (change in Epworth Sleepiness Scale: BCAA group, -5.5 vs. control group, 1.2;  $P<0.05$ ).<sup>145</sup> and nutritional status or sarcopenia.<sup>139,146,147</sup> As for the method of BCAA administration, both taking BCAAs as drugs and eating BCAA-rich foods during

**Table 4.** Summary of clinical studies in BCAA

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcome	Study design
Muto et al. <sup>11</sup> (2005)	Mixed (mainly HCV)	Decompensated cirrhosis	2 years	BCAA (314) Control (308)	62±8 61±9	Higher/longer event-free survival in BCAA group (death, varix rupture, HCC, hepatic failure) (HR, 0.67; 95% CI, 0.49–0.93, P=0.015)	RCT
Marchesini et al. <sup>144</sup> (2003)	Mixed (mainly viral)	Advanced cirrhosis (CTP B or C)	1 year	BCAA (59) Lactalbumin (56) Maltodextrins (59)	59±1 60±1 59±1	Event (death, varix rupture, HCC, hepatic failure) (15.5% vs. 32.1% vs. 27.1%; P=0.037)	RCT
Ichikawa et al. <sup>145</sup> (2010)	Mixed (mainly viral)	Liver cirrhosis	8 weeks	BCAA (12) Control (9)	66.2±8.2 67.4±9.8	Change in ESS (mean, -5.5 vs. 1.2; P<0.05)	RCT
Yamamoto et al. <sup>154</sup> (2005)	Mixed (mainly viral)	Liver cirrhosis	1 hour	BCAA (16) Control (13)	63±8 62±9	Change in cerebral blood flow (PET) (mean, 0.81 vs. 0.75; P<0.05)	RCT
Kawamura et al. <sup>138</sup> (2009)	Mixed (mainly HCV)	Liver cirrhosis with CTP class A	1 year	BCAA (27) Control (23)	62.7±10.08 62.3±7.3	Cirrhosis-related complications (HCC, ascites, varix, HE) (14.8% vs. 30.4%; P=0.043)	RCT
Les et al. <sup>32</sup> (2011)	Mixed (mainly HCV, alcohol)	Liver cirrhosis with episode of HE within 2 months	56 weeks	BCAA (21) Control, maltodextrin (27)	64.1±10.4 62.5±10.4	Recurrence of HE (4.7% vs. 34%; P=0.274)	RCT
Nakaya et al. <sup>139</sup> (2007)	HCV	Liver cirrhosis	3 months	BCAA (19) Control (19)	67±9 67±8	Change in albumin level (mean, 3.2 vs. 3.0; P<0.05)	RCT
Takeshita et al. <sup>29</sup> (2012)	HCV	HCV with insulin resistance	24 weeks	BCAA (14) Control (13)	58.6±2.9 64.2±3.0	HOMA-IR after treatment (mean, 4.5 vs. 5.3; P=0.047)	RCT
Koreeda et al. <sup>149</sup> (2011)	Mixed (mainly HCV)	Liver cirrhosis	6 months	BCAA (17)	68±10	Change in Rmax (mean, 0.23 to 0.25; P=0.059)	Prospective cohort study
Park et al. <sup>142</sup> (2020)	Mixed (mainly alcohol)	Liver cirrhosis with CTP score of 8–10 points	6 months	BCAA (63) Control (61)	60±10 58±11	Higher/longer event-free survival in BCAA group (death, varix rupture, HCC, hepatic failure) (HR, 0.38; 95% CI, 0.22–0.68, P<0.001)	Prospective cohort study
Park et al. <sup>143</sup> (2017)	Mixed (mainly HBV, alcohol)	Liver cirrhosis with CTP score of 8–10 points	6 months	BCAA (166) Control (141)	59±11 60±9	Cirrhotic complication-free survival (median, 19.3 vs. 19.2 months; P=0.973)	Retrospective cohort study

**Table 4.** Continued

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcome	Study design
Hanai et al. <sup>277</sup> (2015)	Mixed (mainly HCV)	Liver cirrhosis patients who were not transplantation candidates	1 year	BCAA (94) Control (36)	64 (28–91) 66 (33–84)	Higher/longer overall survival in BCAA group (log-rank test $P=0.02$ )	Retrospective cohort study
Hanai et al. <sup>148</sup> (2020)	Mixed (mainly HCV, alcohol)	Liver cirrhosis	NA	BCAA (87) Control (436)	69 (59–74) 66 (55–74)	Lower mortality in BCAA group (HR, 0.57; 95% CI, 0.33–0.99, $P=0.046$ )	Retrospective cohort study

Variables are expressed as median (interquartile range) or mean±standard deviation.  
BCAA, branched chain amino acid; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; RCT, randomized controlled trial; CTP, Child-Turcotte-Pugh; ESS, Epworth Sleepiness Scale; PET, positron emission tomography; HE, hepatic encephalopathy; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HBV, hepatitis B virus.

late-night meals were effective.<sup>139,148,149</sup> On the other hand, BCAA administration did not lead to improvements in measures of liver function itself, such as Model for End-stage Liver Disease (MELD) or Child-Turcotte-Pugh (CTP) scores, in many studies.<sup>138,140,142–144</sup> Also, whether or not BCAs can prolong overall survival (OS) differs between studies. Recently, two meta-analyses showed that BCAA supplements had no effect on survival.<sup>150,151</sup>

Although BCAA administration was effective in improving liver function and reducing the risk of decompensation in patients with liver cirrhosis, further prospective studies are still required to discern whether BCAA is effective for patients with non-cirrhosis.

## HE

BCAAs have been reported to be effective in both minimal HE and overt HE (Table 4).<sup>32,151</sup> According to meta-analysis results, BCAA administration improved HE compared to the control group (RR, 1.71; 95% CI, 1.17–2.51).<sup>150</sup> However, in patients with a previous history of HE, BCAA administration had no significant effect on the recurrence of HE (BCAA group, 47% vs. control group, 34%;  $P=0.274$ ).<sup>32</sup> This phenomenon can be explained by that facts that BCAAs reduce ammonia levels<sup>152,153</sup> and increase cerebral blood flow in cirrhotic patients,<sup>154</sup> respectively.

To date, BCAA administration has shown a beneficial effect in patients with HE. However, additional research is needed to determine whether BCAA administration will be effective for decompensation in conditions other than HE, such as variceal bleeding, ascites, and jaundice.

## Safety

Adverse events related to BCAA administration were not reported in most studies, including RCTs. In a Cochrane review of 5 studies,<sup>11,144,155–157</sup> BCAA did not increase the risk of serious adverse events, but they were associated with nausea and diarrhea, although not to a significant degree (RR, 3.39; 95% CI, 0.7–16.46).<sup>151</sup>

## STATIN

### Mechanism of action

Statins are inhibitors of hydroxymethylglutaryl coenzyme

A (HMG-CoA) reductase and are used as lipid-lowering agents by >200 million patients worldwide.<sup>158</sup> The major pharmacologic effect of statin is decreased production of cholesterol precursors and cholesterol biosynthesis, resulting in the prevention of atherosclerosis to reduce cardiovascular and cerebrovascular events and mortality.<sup>23</sup> In addition to their lipid-lowering effect, statins are well known to have other beneficial effects, causing improvements in endothelial function and displaying anti-inflammatory, immunomodulatory, and anti-thrombotic effects (Fig. 2C).<sup>159</sup> Statins interfere with the activation of small GTPases like RhoA and Ras proteins, which can modulate endothelial nitric oxide synthase- and nitric oxide activity.<sup>160</sup> Statins activate PPAR- $\alpha$  and - $\beta$  oxidation, resulting in the reduction of intrahepatic inflammation.<sup>161</sup> Statins can also induce the protection of liver sinusoidal endothelial cells (LSECs) and mediate paracrine endothelial-HSC deactivation through the induction of transcription factor Kruppel-like factor 2.<sup>162</sup> Deactivation of HSCs and stabilization of LSECs lead to the alleviation of hepatic fibrosis and portal pressure.<sup>163</sup> Statins also showed anti-tumor effects in HCC by reducing cell proliferation and tumor cell adhesion.<sup>164</sup>

## Clinically beneficial effects

### NAFLD

Several reports have suggested beneficial effects of statins in NAFLD and NASH (Table 5). Nelson et al.<sup>12</sup> conducted an RCT that investigated the therapeutic effects of simvastatin in biopsy-proven NASH patients. This study included a small number of patients, and there was no significant difference in necroinflammatory activity (mean,  $1.4 \pm 0.5$  vs.  $1.0 \pm 1.4$ ;  $P > 0.05$ ) or fibrosis stage ( $1.50 \pm 0.9$  vs.  $1.0 \pm 1.4$ ;  $P > 0.05$ ) between the simvastatin group and placebo group. However, two cross-sectional studies reported that statin treatment had protective effects against NASH progression. One study enrolled 108 statin users and 1,094 controls who received liver biopsy for suspected NASH.<sup>165</sup> In this study, statin treatment was associated with a reduced risk of steatosis (odds ratio [OR], 0.09; 95% CI, 0.01–0.32,  $P = 0.004$ ), NASH (OR, 0.25; 95% CI, 0.13–0.47,  $P < 0.001$ ), and F2–F4 fibrosis (OR, 0.42; 95% CI, 0.20–0.80;  $P = 0.017$ ) after matching.<sup>165</sup> The other study enrolled 346 diabetes patients with biopsy-proven NAFLD and reported that statin treatment reduced the risk of NASH (OR, 0.57; 95% CI, 0.32–1.01,  $P = 0.055$ ) and F2–F4 fibrosis (OR, 0.47; 95% CI, 0.26–0.84,  $P = 0.011$ ).<sup>166</sup> In addition, another retrospec-

tive cohort study reported that statin treatment significantly reduced the amount of hepatic steatosis from a mean rate of 20.4% at baseline to 11.1% at follow-up ( $P = 0.001$ ), whereas the control group did not experience such a change.<sup>167</sup>

Several prospective cohort studies have reported that statin treatments using pravastatin, atorvastatin, and rosuvastatin have beneficial effects in improving the histologic grade of NAFLD and NASH.<sup>168–171</sup> On the other hand, pitavastatin did not significantly change NAS or fibrosis stage.<sup>172</sup> In a large population-based study, statin treatment reduced the risk of NAFLD development (adjusted OR, 0.66; 95% CI, 0.65–0.67) and fibrosis development (adjusted OR, 0.43; 95% CI, 0.42–0.44). In a study using data from the National Health Information database of South Korea, statin treatment decreased not only the risk of NAFLD occurrence but also the development of fibrosis attributed to NAFLD, regardless of diabetes mellitus.<sup>173</sup> A meta-analysis by Fatima et al.<sup>174</sup> reported that statin treatment reduced the risk of NAFLD development (OR, 0.69; 95% CI, 0.57–0.84;  $I^2 = 36\%$ ;  $P = 0.0002$ ); ALT and GGT levels; and histologic grades with steatosis, inflammation, and fibrosis. There was also a report that statin treatment reduced the HCC risk in patients with NAFLD (HR, 0.47; 95% CI, 0.36–0.60).<sup>175</sup>

Collectively, there is increasing evidence that statin treatment might have protective effects on NAFLD/NASH development and a beneficial effect of histologic improvement for NAFLD/NASH. Therefore, statins can be used in NAFLD and NASH, and they are considered first-line treatments to lower low-density lipoprotein (LDL) cholesterol and prevent atherosclerotic cerebrovascular disease. However, well-organized RCTs are required to establish a therapeutic effect of statin in NAFLD/NASH patients with histologic confirmation, and long-term, large-scale prospective cohort studies are also needed to identify whether statin treatments are associated with liver-related outcomes in patients with NAFLD/NASH.

### Viral hepatitis

Three studies with CHB patients reported another beneficial effect of statin use. According to a large-scale retrospective cohort study including patients with CLD, lovastatin use was associated with lower incidence rates of liver-test abnormalities (incident RR [IRR], 0.28; 95% CI, 0.12–0.55), moderate liver injury (IRR, 0.56; 95% CI, 0.47–0.65), severe liver injury (IRR, 0.50; 95% CI, 0.29–0.81), and the occurrence of liver cirrhosis and liver failure (IRR, 0.29; 95% CI, 0.21–0.38).<sup>176</sup> Anoth-

**Table 5.** Summary of clinical studies in statin

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Nelson et al. <sup>12</sup> (2009)	NAFLD	Biopsy-proven NASH	12 months	Simvastatin 40 mg/day (10) Control (6)	52.6±8.6 52.5±13.0	· Necroinflammatory activity (mean, 1.4 vs. 1.0; $P>0.05$ ) · Fibrosis stage (mean, 1.50 vs. 1.0; $P>0.05$ )	RCT
Dongiovanni et al. <sup>165</sup> (2015)	NAFLD	Patients who underwent liver biopsy for suspected NASH	≥6 months	Statin (107) Control (1,094)	53±10 41±16	· Lower presence of steatosis (OR, 0.09; 95% CI, 0.01–0.32; $P=0.004$ ), NASH (OR, 0.25; 95% CI, 0.13–0.47; $P<0.001$ ), F2–F4 fibrosis (OR, 0.42; 95% CI, 0.20–0.80; $P=0.017$ )* in statin group	Cross-sectional study
Nascimbeni et al. <sup>166</sup> (2016)	NAFLD	Biopsy-proven NAFLD with type 2 DM	NA	Statin (154) Control (192)	55 (48–61) 52 (42–58)	· Lower presence of NASH (OR, 0.57; 95% CI, 0.32–1.01; $P=0.055$ ) and significant fibrosis (OR, 0.47; 95% CI, 0.26–0.84; $P<0.011$ ) in statin group	Cross-sectional study
Ekstedt et al. <sup>167</sup> (2007)	NAFLD	Biopsy-proven NAFLD with elevated ALT and/or AST >41 U/L and/or elevated ALP >106 U/L	10.3–16.3 years	Statin (17) Control (51)	48.7±9.1 46.3±11.8	· Significant reduction of liver steatosis in statin group (20.4% to 11.1%, $P=0.001$ )	Retrospective cohort study
Rallidis et al. <sup>168</sup> (2004)	NAFLD	Biopsy-proven NASH and abnormal liver enzyme	6 months	Pravastatin 20 mg/day (5)	40±8	· Improvement in the grade of inflammation (75%) and steatosis (25%)	Prospective cohort study
Hyogo et al. <sup>169</sup> (2008)	NAFLD	Biopsy-proven NASH with lipidemia	24 months	Atorvastatin 10 mg/day (31)	52.5 (27–68)	· Significant improvement of steatosis grade (1.6 to 0.8; $P<0.001$ ) and NAS (4.1 to 2.9; $P<0.001$ )	Prospective cohort study
Hyogo et al. <sup>172</sup> (2011)	NAFLD	Biopsy-proven NASH and hyperlipidemia	12 months	Pitavastatin 2 mg/day (20)	50.6 (25–75)	· Change of NAS (6.7 to 6.3) and fibrosis stage (2.3 to 2.1)	Prospective cohort study
Nakahara et al. <sup>170</sup> (2012)	NAFLD	Biopsy-proven NASH and hyperlipidemia	24 months	Rosuvastatin 2.5 mg/day (19)	46.3 (20–65)	· Change of NAS (3.89 to 3.44) and fibrosis stage (2.33 to 2.00)	Prospective cohort study
Kargiotis et al. <sup>171</sup> (2015)	NAFLD	Biopsy-proven NASH and metabolic syndrome and lipidemia	12 months	Rosuvastatin 10 mg/day (20)	40.5±5.6	· Complete resolution of NASH (95%)	Prospective cohort study

**Table 5.** Continued

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Lee et al. <sup>278</sup> (2021)	NAFLD	Age ≥20 years who participated the NHIS physical health examination	72 months	NAFLD (164,856) Control (824,280)	41.4±12.4 41.4±12.4	· Reduced risk of NAFLD development in statin group (adjusted OR 0.66; 95% CI, 0.65–0.67)	Retrospective cohort study
Zou et al. <sup>175</sup> (2022)	NAFLD	Diabetes or obesity and ICD-10 codes (K76.0 and K758.1)	1.92 years	Statin (73,385) Control (199,046)	58.0±12.4 50.0±14.9	· Lower risk of HCC development in statin group (HR, 0.47; 95% CI, 0.36–0.60; $P<0.001$ )	Retrospective cohort study
Avins et al. <sup>176</sup> (2008)	Mixed	Patients with evidence of liver disease showing elevated AST or ALT levels or diagnosis of liver disease	28.8 months (12.1–58.2)	Lovastatin (13,491) Control (79,615)	53.9±11.4 47.5±13.6	· Lower incidence of liver function test abnormalities in statin group (incident RR 0.28; 95% CI, 0.12–0.55; $P=NA$ )	Retrospective cohort study
Hsiang et al. <sup>177</sup> (2015)	HBV		1.6 years	Statin (1,176)	58.7±12.4	· Lower HCC development in statin group (subHRR 0.68; 95% CI, 0.48–0.97; $P=0.033$ )	Retrospective cohort study
Huang et al. <sup>178</sup> (2016)	HBV		4.9 years	Control (52,337)	37.6±14.1		
			4.71±3.21	Statin (22,544)	52.87±11.51	· Lower incidence of cirrhosis (RR, 0.433; 95% CI, 0.344–0.515; $P<0.001$ ) and decompensated cirrhosis (RR, 0.468; 95% CI, 0.344–0.637; $P<0.001$ ) in statin group	Retrospective cohort study
Butt et al. <sup>273</sup> (2015)	HCV	Received HCV treatment ≥14 days	>24 months	Statin (3,347) Control (3,901)	53 (49–56) 52 (48–56)	· Higher SVR rate (OR, 1.44; 95% CI, 1.29–1.61; $P<0.0001$ ) in statin group · Cirrhosis development (17.3% vs. 25.2%; $P<0.001$ ) · HCC incidence (1.2% vs. 2.6%, $P<0.01$ )	Retrospective cohort study
Simon et al. <sup>179</sup> (2015)	HCV	Previous non-response to standard interferon therapies and advanced hepatic fibrosis on liver biopsy	3.5 years	Statin (29) Control (514)	54.2±7.2	· Lower risk of fibrosis progression in statin group (unadjusted HR, 0.32; 95% CI, 0.10–0.97; $P=0.048$ ; adjusted HR, 0.31; 95% CI, 0.10–0.97; $P=0.044$ )	Retrospective cohort study

**Table 5.** Continued

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Yang et. el. <sup>180</sup> (2015)	HCV	2,874,031.7 person-years	Statin (29,204) Control (19,765)	Only distribution available	· Incidence rate of cirrhosis (445.5/100,000 person-years vs. 1311.2/100,000 person-years)		Retrospective cohort study
Mohanty et al. <sup>181</sup> (2016)	HCV	2.6 years 1.9 years	Statin (1,323) Control (12,522)	56 (52–60) 54 (50–58)	· Lower risk of decompensation (HR, 0.22; 95% CI, 0.17–0.28) and death (HR, 0.39; 95% CI, 0.34–0.44) before matching in statin group · Lower risk of decompensation (HR, 0.55; 95% CI, 0.39–0.77) and death (HR, 0.56; 95% CI, 0.46–0.69) after matching in statin group		Retrospective cohort study
Abraldes et al. <sup>182</sup> (2009)	Mixed (mainly alcohol)	Liver cirrhosis patients with severe portal HIN defined as HVPG of ≥12 mmHg	30±4 days	Simvastatin (28) Control (27)	58±10 56±10	· Change in HVPG (mean, -8.3% vs. -1.6%; P=0.041)	RCT
Pollo-Flores et al. <sup>183</sup> (2015)	Mixed (mainly HCV)	Cirrhosis with portal HTN	3 months	Simvastatin (14) Control (20)	56.5 (IQR, 8.7) 58.5 (IQR, 13.5)	· Decreased HVPG of at least 20% from baseline or to ≤12 mmHg (55% vs. 0%; P=0.03)	RCT
Abraldes et al. <sup>184</sup> (2016)	Mixed (mainly alcohol)	Diagnosis of liver cirrhosis, and variceal bleeding within the previous 5–10 days, and plan to start standard prophylactic treatment for variceal bleeding	371 days 382 days	Simvastatin (69) Control (78)	57.42±11.31 57.62±10.59	· Higher survival (HR, 0.387; 95% CI, 0.152–0.986; P=0.030) in statin group · Lower rebleeding risk (HR, 0.858; 95% CI, 0.455–1.620, P=0.583) in statin group	RCT
Kumar et al. <sup>185</sup> (2014)	Mixed (mainly HCV)	Biopsy-proven liver cirrhosis on statin therapy at biopsy and for ≥3 months after biopsy	36 months 30 months	Statin (81) Control (162)	59.79±10.91 59.64±10.60	· Lower mortality (HR, 0.53; 95% CI, 0.334–0.856; P=0.01) in statin group · Lower risk of decompensation (HR, 0.58, 0.34–0.98; P=0.04) in statin group	Retrospective cohort study

Variables are expressed as median (interquartile range) or mean±standard deviation.

NALFD, nonalcoholic steatohepatitis; RCT, randomized controlled trial; OR, odds ratio; CI, confidence interval; DM, diabetes mellitus; NA, not available; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; NAS, NALFD activity score; NHIS, National Health Interview Survey; CD-10, International Classification of Diseases; HCC, hepatocellular carcinoma; HR, hazard ratio; RR, risk ratio; HBV, hepatitis B virus; HCV, hepatitis C virus; SVR, sustained viral response; HTN, hypertension; HVPG, hepatic venous pressure gradient; IQR, interquartile range.

\*These data are after matching.

er study by Hsiang et al.<sup>177</sup> reported that statin treatment is associated with a lower risk of HCC development (weighted sub-HR, 0.68; 95% CI, 0.48–0.97;  $P=0.033$ ). Another nationwide cohort study reported that statin use significantly lowered the incidence of cirrhosis (RR, 0.433; 95% CI, 0.344–0.515;  $P<0.001$ ) and decompensated cirrhosis (RR, 0.468; 95% CI, 0.344–0.637;  $P<0.001$ ) compared to that of a non-statin group.<sup>178</sup>

Statins might be associated with a sustained virologic response (SVR) in patients with HCV. Patients treated with statins showed higher SVRs (OR, 1.44; 95% CI, 1.29–1.61;  $P<0.0001$ ) and decreased progression of liver fibrosis and incidence of HCC among CHC patients who received pegylated IFN-based HCV treatment for  $\geq 14$  days.<sup>173</sup> Other retrospective studies also showed that statin treatment is associated with the decreased risk of fibrosis and cirrhosis progression in patients with HCV (Table 5).<sup>179–181</sup>

Despite the positive results in patients with HBV or HCV, the clinical relevance of statins might be limited mainly due to the weakness of a retrospective study design. Therefore, it is premature to routinely prescribe statins for clinical benefit before well-designed prospective studies are available.

### Liver cirrhosis

Two RCTs showed that simvastatin significantly reduced the hepatic venous pressure gradient (HVPG) and improved hepatic perfusion in patients with cirrhosis.<sup>182,183</sup> In another RCT, simvastatin failed to reduce the risk of rebleeding (HR, 0.858; 95% CI, 0.455–1.620;  $P=0.583$ ) but prolonged survival (HR, 0.387; 95% CI, 0.152–0.986,  $P=0.030$ ) in cirrhotic patients with variceal bleeding.<sup>184</sup> A retrospective study reported that statin use was associated with lower risks of mortality and decompensation (Table 5).<sup>185</sup>

Statins have potential beneficial effects in cirrhosis patients, but their long-term beneficial effects are limited. In addition, safety is also a concern, especially in patients with cirrhosis. Further prospective long-term follow-up data are required to confirm the beneficial effect of statins in patients with cirrhosis.

### Safety

There are several concerns surrounding the adverse effects of statins, which include myopathy, a risk of type 2 diabetes development, deterioration of neurological and neurocognitive conditions, renal toxicity, and hepatotoxicity.<sup>186</sup> Amino-

transferase elevation was found in up to 2% of patients in early clinical trials.<sup>187</sup> The mechanism of statin-induced hepatotoxicity is not clearly defined: mitochondrial dysfunction by oxidative stress, increase of ROS synthesis, immune-allergic reactions, and lactic acidosis may be potential mechanisms (Fig. 2C).<sup>188</sup> However, most cases of aminotransferase elevation are mild and transient. Although the increase in liver enzyme levels depends on the statin dose, the incidence of AST or ALT level  $>3$  times of the upper normal range is 0–1.8%, and clinically significant acute liver injury or fulminant hepatic failure is very rare.<sup>189</sup> In a previous study of patients with cirrhosis, two cases of severe rhabdomyolysis (2.8%) developed in the simvastatin group.<sup>184</sup> As even a small dose of statin than the usual dose can lead to adverse events, including rhabdomyolysis, in patients with decompensated cirrhosis, the administration of statins to patients with decompensated cirrhosis or acute liver failure should be avoided. Further studies for determining the safety dose of statin are required in patients with advanced liver disease.

## PROBIOTICS

### Mechanism of action

The gut microbiota has been considered a novel environmental factor involved in the pathophysiology of liver diseases.<sup>190</sup> A growing body of evidence points towards the suggestion that intestinal dysbiosis contributes to impaired barrier function of the intestinal mucosa.<sup>191</sup> Enhanced intestinal permeability allows bacterial metabolites such as LPS to reach the liver.<sup>192,193</sup> LPS trigger inflammation and insulin resistance by activating toll-like receptor (TLR)-4 and initiating pro-inflammatory cascades.<sup>194</sup> Overall, intestinal dysbiosis, bacterial translocation, and TLR-4 activation lead to increased hepatic fat accumulation, prompting the development of NAFLD and progression to NASH.<sup>195</sup>

Modulation of the gut microbiota would represent an attractive target for therapeutic interventions in NAFLD subjects. *In vivo* studies have demonstrated the therapeutic effects of probiotics on NAFLD (Fig. 2D).<sup>25</sup> In high-fat diet-fed murine models, probiotics induce *Bifidobacterium* abundance and a more beneficial composition of gut microbiota.<sup>196</sup> Ingestion of *Lactobacillus* also ameliorates the progression of NAFLD in murine models with a Western diet.<sup>197</sup>

In terms of the gut-liver-brain axis, conditions of altered communication between the gut microbiota and the brain, such as dysbiosis, leaky gut, metabolic endotoxemia, and brain changes, may induce the development of HE in patients with liver cirrhosis.<sup>190,198</sup> Conversely, probiotics can reduce ammonia absorption by decreasing the urease activity of gut bacteria in the intestinal lumen and the intestinal pH.<sup>199</sup>

## Clinically beneficial effects

### NAFLD

Probiotics are live bacteria that intend to improve the “good” gut microbiota by competitive colonization and acidification of the intestinal lumen.<sup>200</sup> *Lactobacillus* and *Bifidobacterium* are the most commonly used species in probiotics. Seven representative RCTs have investigated the therapeutic effect of probiotics in patients with NAFLD (Table 6).<sup>201-207</sup> Although heterogeneities exist in terms of the dosing and type of probiotics and the treatment period in these RCTs, probiotic intervention could be related to diminishing liver steatosis assessed by imaging modalities compared to placebo.<sup>202,204-207</sup> In addition, improvements in several biochemical markers, including ALT, AST, and GGT, and metabolic profiles such as total and LDL cholesterols were also observed in probiotic groups.<sup>201-207</sup> Unfortunately, these studies included small numbers of patients, and few of them examined the effect of probiotics on histologic markers of NASH.

Synbiotics are a combination of advantageous gut bacteria (probiotics) and non-digestible fibers that help good bacteria to grow (prebiotics). Most RCTs evaluating the effect of synbiotics in NAFLD demonstrated significant reductions in liver enzymes and steatosis as measured by ultrasound.<sup>13,30,208-211</sup> Interestingly, an European RCT including 75 patients with NASH fed a low-fat/low-calorie diet reported that end-of-study liver stiffness as measured by transient elastography was significantly lower in patients treated with 12 weeks of synbiotics compared to the control group (mean,  $5.2 \pm 0.2$  vs.  $5.9 \pm 0.2$  kPa;  $P < 0.05$ ), with significant differences in the serum cholesterol (mean,  $5.4 \pm 0.2$  vs.  $6.0 \pm 0.2$  mmol/L;  $P < 0.05$ ) and BMI (mean,  $21.1 \pm 0.6$  vs.  $23.9 \pm 0.6$  kg/m<sup>2</sup>;  $P < 0.05$ ).<sup>210</sup> Synbiotic supplementation was also associated with a greater reduction in fibrosis among Iranian lean NAFLD subjects who underwent lifestyle modification (mean change  $\pm$  standard error,  $-1.71 \pm 0.25$  vs.  $-0.71 \pm 0.18$  kPa;  $P < 0.001$ ).<sup>208</sup> A recent U.K. phase

II RCT showed that the administration of synbiotics without lifestyle intervention altered the fecal microbiome, with increased proportions of *Bifidobacterium* and *Faecalibacterium* and reductions in *Oscillibacter* and *Alistipes*, compared with baseline.<sup>33</sup> However, such did not significantly improve magnetic resonance imaging-based liver fat content or indirect markers of liver fibrosis. Finally, a recent meta-analysis involving 28 clinical trials enrolling 1,555 patients with NAFLD revealed that syn-/probiotic therapy had beneficial effects on BMI (mean difference, -1.46; 95% CI, -2.44 to -0.48;  $I^2 = 97\%$ ;  $P < 0.001$ ), ALT (mean difference, -13.40; 95% CI, -17.03 to -9.77;  $I^2 = 94\%$ ;  $P < 0.001$ ), AST (mean difference, -13.54; 95% CI, -17.86 to -9.22;  $I^2 = 96\%$ ;  $P < 0.001$ ), HOMA-IR (mean difference, -0.42; 95% CI, -0.73 to -0.12;  $I^2 = 79\%$ ;  $P = 0.007$ ), and total cholesterol (mean difference, -15.38; 95% CI, -26.50 to -4.25;  $I^2 = 93\%$ ;  $P = 0.007$ ) levels.<sup>212</sup>

Collectively, probiotic supplementation can be used as a complementary approach for managing patients with NAFLD, especially in combination with lifestyle interventions. However, the identification of appropriate bacterial strains and proper duration of treatment as well as potential interactions with other targeted agents require further investigation.

### Liver cirrhosis

Data exist on the beneficial role of probiotics in treating patients with liver cirrhosis, especially HE. The effect of probiotics in secondary prophylaxis was evaluated in an RCT involving 130 Indian patients who had recovered from HE.<sup>213</sup> The probability of hospitalization for HE was significantly lower in patients treated with 6 months of probiotics compared to others (19.7% vs. 42.2%; HR, 0.45; 95% CI, 0.23–0.87;  $P = 0.02$ ). Significant improvements in CTP score and MELD scores from baseline were observed only in the probiotic group (median [interquartile range]: 8.81 [7.98–9.64] to 7.19 [6.63–7.75],  $P < 0.001$  for CTP score; 17.00 [13.60–20.40] to 13.25 [11.88–14.62],  $P < 0.26$  for MELD score). Another Indian RCT including 160 patients with minimal HE indicated that probiotics could effectively prevent overt HE (1.2% vs. 19%; HR for control vs. probiotic group, 2.1; 95% CI, 1.31–6.53;  $P < 0.05$ ).<sup>214</sup> A recent meta-analysis including 14 trials compared probiotics to placebo/no treatment in patients with HE demonstrated that the probiotics group had a significant lower prevalence of incomplete resolution of HE (RR, 0.67; 95% CI, 0.56–0.79) and development of overt HE (RR, 0.29;

**Table 6.** Summary of clinical studies in probiotics

Study	Etiology	Inclusion criteria	Treatment	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Wong et al. <sup>202</sup> (2013)	NAFLD	Biopsy-proven NASH	Mixture of <i>L. plantarum</i> , <i>L. delbrueckii</i> , <i>L. acidophilus</i> , <i>L. rhamnosus</i> , and <i>B. bifidum</i>	6 months	Probiotics (10) Usual care (10)	42±9 55±9	· Decreased liver fat contents in probiotics (22.6% to 14.9%; $P=0.034$ ) · Decreased AST level in probiotics (mean, 83.3 to 46.1; $P=0.008$ )	RCT
Shayakh et al. <sup>205</sup> (2013)	NAFLD	Biopsy-proven NASH on metformin	<i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Streptococcus</i>	6 months	Probiotics + metformin (31) Placebo + metformin (32)	41.5±12.7 55±9	· Decreased ALT and AST levels (mean, 133.7 to 45.2 vs. 123.1 to 44.2; $P<0.001$ ) · Reduction in grade of hepatic steatosis measured by US	RCT
Nabavi et al. <sup>203</sup> (2014)	NAFLD	NAFLD on US	<i>L. acidophilus</i> and <i>B. lactis</i>	8 weeks	Probiotic yoghurt (36) No probiotics (15)	42.74±8.72 44.05±8.14	· Lower AST, ALT, total cholesterol, and LDL-cholesterol levels after treatment in probiotic yogurt groups than control ( $P<0.05$ )	RCT
Abdel Monem <sup>201</sup> (2017)	NAFLD	Biopsy-proven NASH	<i>L. acidophilus</i>	1 month	Probiotics (15) No probiotics (15)	44.20±5.51 44.33±5.62	· Decreased ALT level in probiotics (mean, 83.3 to 46.1; $P<0.001$ ) · Decreased AST level in probiotics (mean, 57.1 to 38.2; $P=0.03$ )	RCT
Manzhalii et al. <sup>210</sup> (2017)	NAFLD	NASH fed a low-fat/low-calorie diet	<i>L. casei</i> , <i>L. rhamnosus</i> , <i>L. bulgaris</i> , <i>B. longum</i> , <i>S. thermophilus</i> and oligofructose	12 weeks	Probiotic cocktail (38) No probiotics (37)	44.3±1.5 43.5±1.3	· Greater reduction in AST and ALT levels in symbiotics than placebo ( $P<0.05$ ) · Greater reduction in the fibrosis score by TE in symbiotics than placebo ( $P<0.05$ )	RCT
Kobylak et al. <sup>204</sup> (2018)	NAFLD	Diabetes	<i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Propionibacterium</i>	8 weeks	Probiotics (30) Placebo (28)	53.4±9.55 57.3±10.5	· Decreased AST and GGT levels in probiotics ( $P<0.05$ ) · Decreased fatty liver index in probiotics (84.33 to 78.73; $P<0.001$ )	RCT
Ahn et al. <sup>207</sup> (2019)	NAFLD	Obesity and liver fat >5% on proton density fat fraction	<i>Lactobacillus</i> , <i>Pediococcus</i> , <i>Bifidobacterium</i>	12 weeks	Probiotics (30) Placebo (35)	41.7±12.49 44.71±13.31	· Decreased liver fat contents (mean 16.3% to 14.1%; $P=0.032$ ) · Greater reduction in the triglyceride level in probiotics than placebo ( $P=0.003$ )	RCT
Duseja et al. <sup>206</sup> (2019)	NAFLD	Biopsy-proven NAFLD	<i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Streptococcus</i>	1 year	Oral multistrain probiotic (19) Placebo (20)	38±10 33±6	· Greater reduction in ALT level in probiotics than placebo ( $P=0.046$ ) · Greater reduction in the NAS and hepatic fibrosis in probiotics than placebo ( $P<0.05$ )	RCT

**Table 6.** Continued

Study	Etiology	Inclusion criteria	Treatment	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Malaguarnera et al. <sup>30</sup> (2012)	NAFLD	Abnormal serum aminotransferase levels	<i>B. longum</i> and oligofructose	24 weeks	Symbiotics + LSM (33) Placebo + LSM (33)	46.9±5.4 46.7±5.7	· Lower AST and LDL-cholesterol levels after treatment in symbiotics than placebo ( $P<0.05$ ) · Greater reduction in HOMA-IR and NASH activity score in symbiotics than placebo ( $P<0.05$ )	RCT
Eslamparast et al. <sup>13</sup> (2014)	NAFLD	NAFLD on US with ALT >60 IU/L for 6 months	Combination of <i>L. casei</i> , <i>L. rhamnosus</i> , <i>S. thermophilus</i> , <i>B. breve</i> , <i>L. acidophilus</i> , <i>B. longum</i> , <i>L. bulgaricus</i> and oligofructose	28 weeks	Symbiotic capsule (26) Placebo (26)	46.35±8.8 45.69±9.5	· Lower AST, ALT and GGT levels after treatment in symbiotics than placebo ( $P<0.001$ ) · Greater reduction in the fibrosis score by TE (mean, 9.36 to 6.38 vs. 7.92 to 7.16; $P<0.001$ )	RCT
Asgharian et al. <sup>219</sup> (2016)	NAFLD		Combination of <i>L. casei</i> , <i>L. rhamnosus</i> , <i>S. thermophilus</i> , <i>B. breve</i> , <i>L. acidophilus</i> , <i>B. longum</i> , <i>L. bulgaricus</i> and oligofructose	8 weeks	Symbiotics (40) Placebo (40)	46.57±1.7 47.78±1.7	· Decreased grade of steatosis on US in symbiotics ( $P<0.005$ )	RCT
Mofidi et al. <sup>208</sup> (2017)	NAFLD	BMI ≤25	Combination of <i>L. casei</i> , <i>L. rhamnosus</i> , <i>S. thermophilus</i> , <i>B. breve</i> , <i>L. acidophilus</i> , <i>B. longum</i> , <i>L. bulgaricus</i> and oligofructose	28 weeks	Symbiotics (21) Placebo (21)	40.09±11.44 44.61±10.12	· Greater reduction in AST and fasting glucose levels in symbiotics than placebo ( $P<0.05$ ) · Greater reduction in the fibrosis score and steatosis by TE in symbiotics than placebo ( $P<0.001$ )	RCT
Sayari et al. <sup>211</sup> (2018)	NAFLD	Taking sitagliptin	<i>Lactobacillus</i> , <i>Bifidobacterium</i> , <i>Streptococcus</i> and fructooligosaccharide	16 weeks	Symbiotics + sitagliptin (70) Placebo + sitagliptin (68)	42.48±11.41 43.42±11.65	· Greater reduction of glucose, AST, total cholesterol, and LDL-cholesterol levels in symbiotics than placebo ( $P<0.05$ )	RCT
Scorletti et al. <sup>33</sup> (2020)	NAFLD	NAFLD diagnosed by histologic confirmation or imaging evidence of liver fat	<i>Bifidobacterium</i> and fructooligosaccharide	10–14 months	Symbiotic agents (55) Placebo (49)	50.2±12.4 51.6±13.1	· No significant difference in MRS-based liver fat reduction between groups	RCT

Variables are expressed as mean±standard deviation.

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; AST, aspartate aminotransferase; RCT, randomized controlled trial; ALT, alanine aminotransferase; US, ultrasonography; LDL, low-density lipoprotein; TE, transient elastography; GGT, γ-glutamyl transferase; LSM, lifestyle modification score; NAS, NAFLD activity score; BMI, body mass index; MRS, magnetic resonance spectroscopy.

95% CI, 0.16–0.51).<sup>215</sup> The plasma ammonia level was also lower for probiotics-treated patients (mean difference, -8.29 µmol/L; 95% CI, -13.17 to -3.41). However, no difference in mortality was observed (RR, 0.58; 95% CI, 0.23–1.44). Overall, all these studies hold the promise that manipulation of intestinal microbiota may be helpful for the management of HE.

### Safety

Probiotics and synbiotics are inexpensive nutritional supplements that are widely available worldwide. Although most clinical trials focused on the beneficial effect of probiotics rather than their safety,<sup>216</sup> probiotics may be safe in immunocompetent adults based on a history of safe use of probiotics in foods. However, probiotics have been associated with a higher risk of bacterial or fungal infection in neonates, infants, and critically ill patients.<sup>217,218</sup> In terms of patients with liver disease, all studies reviewed here reported no significant safety issues with the clinical use of supplementation with probiotic microorganisms in this setting.

## VITAMIN E

### Mechanism of action

Vitamin E (tocopherol) shows antioxidant activity by scavenging ROS and nitrogen species.<sup>219</sup> Vitamin E also increases the action of antioxidative enzymes like superoxide dismutase, catalase, and glutathione peroxidase (Fig. 1).<sup>220,221</sup> In addition to its antioxidative effect, vitamin E has anti-fibrotic, anti-inflammatory, and anti-apoptotic effects.<sup>222-224</sup> Vitamin E supplementation inhibits the activation and proliferation of HSCs in acute damage by CCl<sub>4</sub>.<sup>224</sup> Vitamin E shows an anti-inflammatory effect through inhibiting cyclo-oxygenase-2 and 5-lipoxygenase-mediated eicosanoids and suppressing NF-κB and Janus kinase (JAK)-signal transducer and activator of transcription protein (STAT) 6 or JAK/STAT3 pathways.<sup>222</sup> Vitamin E also inhibits apoptosis by decreasing the proapoptotic proteins.<sup>223</sup>

### Clinically beneficial effects

#### NAFLD

In NAFLD, oxidative stress plays a crucial role in the progression to NASH.<sup>225</sup> Therefore, vitamin E, an antioxidant, has

recently been studied a lot in NAFLD. These studies varied in the duration (3 months to 2 years) and dose (100–800 IU/mL) of vitamin E used and are summarized in Table 7. Vitamin E monotherapy or in combination with other agents significantly improved liver enzymes.<sup>34,87,226-231</sup> In an RCT comparing pioglitazone, vitamin E, and placebo, ALT was significantly decreased in the vitamin E treatment group compared to placebo (mean, -37.0 vs. -20.1;  $P=0.001$ ).<sup>14</sup> Vitamin E treatment significantly improved the hepatic steatosis assessed by ultrasound compared to placebo (34.9% vs. 18.2%;  $P=0.038$ ).<sup>226</sup> Pervez et al.<sup>230</sup> reported that vitamin E supplementation for 12 weeks significantly reduced the fatty liver index (FLI) score compared to placebo (mean change, -12.82 vs. -3.86;  $P<0.001$ ). Aller et al.<sup>232</sup> showed that treatment with a combination of vitamin E and silymarin for 3 months significantly decreased the FLI (from 86.2 to 76.9;  $P<0.05$ ). Vitamin E treatment also improved parameters of the fibrotic burden, as reflected by non-invasive fibrosis surrogates, such as the ratio of AST to platelets score (mean, 0.55 to 0.4;  $P<0.001$ )<sup>34</sup> and NAFLD fibrosis score (mean, -1.6 to -2.1;  $P<0.05$ ).<sup>232</sup> Vitamin E treatment also decreased inflammatory cytokines (IL-6 change: mean, -3.42 vs. -1.56 pg/mL;  $P<0.001$  and TNF-α change: mean, -3.26 vs. -1.15 pg/mL;  $P=0.001$ )<sup>230</sup> or chemokines (CCL-2/monocyte chemo-attractant protein 1: from mean 289 to 131;  $P<0.05$ )<sup>227</sup> and improved insulin resistance (HOMA-IR change: mean, -0.52 vs. -0.13;  $P<0.001$ ).<sup>230</sup> In addition, vitamin E treatment with other agents improved the serum level of adipokine, increasing adiponectin (mean change, +3,808 in UDCA/vitamin E vs. -1,626 in UDCA/placebo vs. -687 ng/mL in placebo/placebo;  $P<0.03$ )<sup>55</sup> and decreasing leptin (mean change, -0.48 vs. 2.54;  $P<0.05$ ) concentrations.<sup>231</sup>

There have been studies showing that vitamin E treatment led to histologic improvement in NAFLD. Harrison et al.<sup>233</sup> reported that treatment with a combination of vitamin E (1,000 IU/day) and vitamin C (1,000 IU/day) for 6 months significantly improved the hepatic fibrosis score ( $P=0.002$ ) in histologically proven NASH, while there was no significant change in inflammation score ( $P>0.05$ ). Dufour et al.<sup>54</sup> showed that treatment with a combination of vitamin E (800 IU/day) and UDCA (12–15 mg/kg/day) for 6 months significantly improved hepatic steatosis ( $P<0.05$ ), while there was no significant change in inflammation or fibrosis. In the PIVENS (Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis) trial of pioglitazone or vitamin E for the treatment of bi-

**Table 7.** Summary of clinical studies in vitamin E

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Harrison et al. <sup>233</sup> (2003)	NAFLD	Histologic diagnosis of NASH	6 months	Vitamin E 1,000 IU/day and C 1,000 mg/day (23) Placebo (22)	50.2 52.5	· Improvement in fibrosis score (47.8% vs. 40.9%; $P=0.005$ ) · No changes in inflammation ( $P>0.05$ ) · ALT improvement ( $P=0.007$ )	RCT
Sanyal et al. <sup>279</sup> (2004)	NAFLD	Non-diabetic, non-cirrhotic	6 months	Vitamin E 400 IU/day (10) Vitamin E 400 IU/day + pioglitazone 30 mg/day (10)	46±13 47±12	· Vitamin E: Improvement in steatosis ( $P=0.02$ ), ballooning ( $P=0.055$ ) and portal fibrosis ( $P>0.05$ ) · Vitamin E + pioglitazone: Improvement in steatosis ( $P=0.002$ ), ballooning ( $P=0.01$ ), and portal fibrosis ( $P>0.05$ ) · Comparison between the two groups: steatosis ( $P<0.05$ ), ballooning ( $P>0.05$ ), portal fibrosis ( $P>0.05$ )	RCT
Ersöz et al. <sup>280</sup> (2005)	NAFLD	Histologically proven NAFLD	6 months	Vitamin E 600 IU/day and C 500 mg/day (28) UDCA 10 mg/kg/day (29)	46.3±9.4 47.9±10.6	· ALT change (IU/L) (mean, 91.9 to 39.1, $P<0.05$ vs. 93.7 to 49.1, $P<0.05$ ; $P>0.05$ )	Open-label RCT
Dufour et al. <sup>54</sup> (2006)	NAFLD	Histologic diagnosis of NASH	6 months	UDCA 12–15 mg/kg/day + vitamin E 800 IU/day (15) UDCA 12–15 mg/kg/day + placebo (18) Placebo + placebo (15)	46±14 47±12 44±14	· Decrease in AST and ALT levels (IU/L) (UDCA + vitamin E vs. placebo, $P<0.05$ ; UDCA vs. placebo, $P>0.05$ ) · Improvement in steatosis (UDCA + vitamin E vs. placebo, $P<0.05$ ; UDCA vs. placebo, $P>0.05$ ) · No improvement in inflammation and fibrosis ( $P>0.05$ )	Double-blinded RCT
Balmer et al. <sup>55</sup> (2009)	NAFLD	Histologic diagnosis of NAFLD	2 years	UDCA 12–15 mg/kg/day + vitamin E 800 IU/day (14) UDCA 12–15 mg/kg/day + placebo (14) Placebo + placebo (13)	47±14 47±12 46±13	· Adiponectin level change (ng/ml) (mean, +3,808 vs. -1,626 vs. -687, $P<0.03$ )	Double-blinded RCT
Sanyal et al. <sup>14</sup> (2010)	NAFLD	Histologic diagnosis of NASH without diabetes	96 weeks	Pioglitazone 30 mg/day (80) Vitamin E 800 IU/day (84) Placebo (83)	47.0±12.6 46.6±12.1 45.4±11.2	· Improvement in NASH (vitamin E 43% vs. placebo 19%, $P=0.001$ ; pioglitazone 34% vs. placebo 19%, $P=0.04$ ) · Improvement in fibrosis (vitamin E 41% vs. placebo 31%, $P=0.24$ ; pioglitazone 44% vs. placebo 31%, $P=0.12$ ) · Changes in serum aminotransferase level (IU/L) (mean, vitamin E -37.0 vs. placebo -20.1, $P=0.001$ ; pioglitazone -40.8 vs. placebo -20.1, $P<0.001$ )	Double-blinded RCT

**Table 7.** Continued

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Aller et al. <sup>232</sup> (2015)	NAFLD	Histologic diagnosis of NAFLD	3 months	Silymarin 1,080.6 mg/day + vitamin E 72 mg/day (18) Control (18)	47.4±11.2	• Decrease in fatty liver index (mean, 86.2 to 76.9; $P<0.05$ ) vs. 85.2 to 77.5; $P<0.05$ • Decrease in NFS (mean, -1.6 to -2.1; $P<0.05$ vs. -1.0 to -1.5; $P<0.05$ )	RCT
Parikh et al. <sup>34</sup> (2016)	NAFLD	Non-diabetic, non-cirrhotic	52 weeks	Vitamin E 800 IU/day (100) UDCA 600 mg/day (150)	43.75±3.5 40.19±2.9	• ALT normalization (14% vs. 19%; $P=0.2$ ) • ALT reduction (56% vs. 63%; $P=0.2$ ) • NFS reduction (44% vs. 47%; $P=0.69$ )	Open-label RCT
Polyzos et al. <sup>229</sup> (2017)	NAFLD	Histologic diagnosis of NASH	52 weeks	Vitamin E 800 IU/day + spironolactone 25 mg/day (14)	54.9±1.8	• ALT reduction (IU/L) (43.5 to 40.0; $P>0.05$ ) vs. 66.0 to 42.1; $P>0.05$ • NAFLD liver fat score reduction ( $P=0.028$ ) vs. $P=0.61$ )	Open-label RCT
Bril et al. <sup>237</sup> (2019)	NAFLD	Histologic diagnosis of NASH and type 2 diabetes	18 months	Vitamin E 800 IU/day (36) Vitamin E 800 IU/day + pioglitazone 30–45 mg/day (37) Placebo (32)	60±9 60±6 57±11	• Reduction of NAS (vitamin E 31% vs. placebo 19%; $P=0.26$ ; vitamin E + pioglitazone 54% vs. placebo 19%; $P=0.003$ ) • Resolution of NASH (vitamin E 33% vs. placebo 12%; $P=0.04$ ; vitamin E + pioglitazone 43% vs. placebo 12%; $P=0.005$ ) • Fibrosis change (vitamin E 50% vs. placebo 30%; $P=0.09$ ; vitamin E + pioglitazone 52% vs. placebo 30%; $P=0.07$ )	Double-blinded RCT
Fouda et al. <sup>227</sup> (2021)	NAFLD	Histologic diagnosis of NASH	3 months	Vitamin E 800 IU/day (34) UDCA 500 mg/day (34) Pentoxifylline 800 mg/day (34)	44.8±9.7 43.4±11 45.2±11	• ALT reduction ( $P<0.05$ ) • Inflammatory cytokine reduction (IL-6, CCL-2/MCP-1) ( $P<0.05$ )	Single-blind RCT
Kedariisetty et al. <sup>228</sup> (2021)	NAFLD	Histologic diagnosis of NASH	1 year	Vitamin E 800 IU/day (33) Vitamin E 800 IU/day + pentoxifylline 1,200 mg/day (36)	35 (16–64) 40 (20–64)	• ALT reduction (IU/L) (mean 85.5 to 28 vs. 97 to 24; $P=0.23$ ) • NAS change (mean 4.3 to 3.1 vs. 5 to 2.8; $P=0.45$ ) • Fibrosis stage change (mean 1.7 to 1.7 vs. 2.1 to 1.0; $P=0.004$ ) • Insulin change (mU/L) (mean 12.4 to 10.8 vs. 12.9 to 7.6; $P=0.048$ ) • TNF- $\alpha$ change (pg/ml) (mean 7.14 to 3.83 vs. 7.85 to 1.59; $P=0.001$ )	Open-label RCT

**Table 7.** Continued

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Groenbaek et al. <sup>248</sup> (2006)	HCV	Elevated ALT	6 months	Vitamin C 500 mg/day + vitamin E 945 IU/day + selenium 200 µg/day (12) Placebo (11)	45 (33–53)	· Change in serum ALT (IU/L) (mean, -8 vs. -6; $P=0.60$ ) · Change in HCV RNA ( $\log_{10}$ eqv/L) (mean, 0.17 vs. 0.41; $P=0.24$ )	Double-blinded RCT
Marotta et al. <sup>246</sup> (2007)	HCV	Cirrhosis, genotype 1, and elevated ALT	6 months	Vitamin E 900 IU/day (25) Fermented papaya preparation 9 g/day (25) Control (10)	62 (54–75)	· Improvement of redox status, GSH, GSSG, GSH/GSSG and MDA; vitamin E ( $P<0.05$ ) and FPP ( $P<0.05$ )	RCT
Bunchoornvatkul et al. <sup>249</sup> (2014)	HCV	Genotype 3	12 weeks	Vitamin E 800 IU/day (19) Placebo (18)	48.8±8.3 49.5±8.6	· Decrease in serum ALT (mean, 105.1 to 96.5; $P=0.260$ vs. 107.5 to 120.4) · ALT responder (57.8% vs. 29.4%; $P=0.106$ )	Double-blinded RCT
Malaguarnera et al. <sup>245</sup> (2015)	HCV	Received PegIFN-α2b + ribavirin	12 months	Silybin 47 mg/day + vitamin E 15 mg/day + phospholipid 97 mg/day (32) Placebo (32)	46.4±6.9	· Decrease in ALT level (IU/L) (mean, 170.2 to 36.9, $P<0.001$ vs. 161.6 to 69.2, $P<0.001$ ; $P<0.001$ ) · Decrease in viremia ( $10^6$ IU/mL) (mean, 5.32 to 1.67, $P<0.05$ vs. 5.4 to 3.24, $P<0.001$ ; $P<0.05$ ) · Decrease in TGF-β (ng/mL) (mean, 54.2 to 32.8, $P<0.05$ vs. 51.8 to 45.2, $P<0.05$ ; $P<0.05$ ) · Decrease in PIIINP (ng/mL) (mean, 43.8 to 33.4, $P<0.001$ vs. 44.7 to 39.8, $P<0.05$ ; $P<0.05$ ) · Decrease in TIMP-1 (ng/mL) (mean, 480.2 to 310.6, $P<0.001$ vs. 487.2 to 421.0, $P<0.001$ ; $P<0.001$ )	Double-blinded RCT
Andreone et al. <sup>251</sup> (2001)	HBV	Positive HBV DNA and raised ALT (1.5×ULN)	3 months	Vitamin E 600 IU/day (15) Control (17)	37±15 42±14	· ALT normalization (47% vs. 6%; $P=0.011$ ) · Negative HBV DNA (53% and 18%; $P=0.039$ ) · Complete response (47% vs. 0%; $P=0.0019$ )	RCT

Variables are expressed as median (interquartile range) or mean±standard deviation.

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ALT, alanine aminotransferase; UDCA, ursodeoxycholic acid; AST, aspartate aminotransferase; NFS, NAFLD fibrosis score; IL-6, interleukin-6; CCL2, C-C motif ligand 2; MCP, monocyte chemoattractant protein; NAS, NAFLD activity score; TNF, tumor necrosis factor; HCV, hepatitis C virus; GSH, glutathione; GSSG, glutathione disulfide; MDA, malondialdehyde; FPP, fermented papaya preparation; PegIFN, pegylated interferon; TGF, transforming growth factor; PIIINP, pro-collagen III N-terminal peptide; TIMP, tissue inhibitor of metalloproteinase; HBV, hepatitis B virus; ULN, upper limit of normal.

opsy-confirmed NASH patients without diabetes, vitamin E treatment (800 IU/day) for 96 weeks, compared to placebo, significantly improved the inflammation (43% vs. 19%;  $P=0.001$ ).<sup>14</sup> Histologic analysis revealed that vitamin E treatment, compared to placebo, reduced the steatosis (54% vs. 31%;  $P=0.005$ ), lobular inflammation (54% vs. 35%;  $P=0.02$ ), and hepatocellular ballooning (50% vs. 29%;  $P=0.01$ ), but fibrosis was not significantly improved (41% vs. 31%;  $P=0.24$ ).<sup>14</sup> Based on the PIVENS trial, guidelines for NAFLD of major societies recommend vitamin E as a treatment in non-diabetic adults with biopsy-proven NASH.<sup>4,234-236</sup> Recently, Bril et al.<sup>237</sup> also reported that vitamin E (800 IU/day) for 18 months achieved a resolution of NASH (33% vs. 12%;  $P=0.04$ ) compared to placebo in adult patients with diabetes and biopsy-proven NASH. In this study, the improvement of fibrosis was not statistically significant (50% vs. 30%;  $P=0.09$ ), but it showed a trend toward a higher rate in vitamin E treatment.<sup>237</sup>

A meta-analysis of histological changes after vitamin E treatment in adult NASH patients revealed significant improvements in steatosis, lobular inflammation, and hepatocellular ballooning.<sup>238,239</sup> However, the results of meta-analyses on fibrosis changes after vitamin E were not consistent. Said and Akhter<sup>239</sup> reported that vitamin E treatment did not significantly improve fibrosis ( $P=0.09$ ), while Vadarlis et al.<sup>238</sup> reported that vitamin E treatment significantly improved fibrosis ( $P=0.005$ ). Meanwhile, a network analysis of studies that assessed the effect of different pharmacological interventions on NASH demonstrated that vitamin E achieved a significant fibrosis improvement compared to placebo (OR, 1.72; 95% CI, 1.04–2.85).<sup>240</sup> Therefore, vitamin E may be an effective treatment in biopsy-proven NASH by improving the steatosis and inflammation. Considering the conflicting results on fibrosis, however, further studies are needed to determine whether vitamin E would improve hepatic fibrosis.

### Viral hepatitis

HCV infection is associated with systemic oxidative stress, which is characterized by increased ROS and nitrogen species and decreased antioxidants.<sup>241</sup> Oxidative stress in HCV patients occurs in the early phase of disease<sup>241,242</sup> and is associated with the progression of fibrosis and development of HCC.<sup>242,243</sup> Because of the association between HCV infection and oxidative stress, vitamin E has been studied in HCV patients as an antioxidant (Table 7). In a double-blinded RCT

with HCV patients refractory to IFN- $\alpha$  therapy by von Herbay et al.,<sup>244</sup> vitamin E treatment (800 IU/day) for 12 weeks significantly lowered serum ALT levels (mean ALT change, -22 vs. +1;  $P<0.001$ ) compared to placebo. Malaguarnera et al.<sup>245</sup> showed that addition of a complex of antioxidants, including vitamin E (15 mg/day), silybin (47 mg/day), and phospholipids (97 mg/day), to IFN- $\alpha$ 2b plus ribavirin led to a significantly greater reduction in viral load (mean,  $-3.65 \times 10^6$  vs.  $-2.16 \times 10^6$  IU/mL;  $P<0.05$ ) and serum levels of hepatic fibrosis markers, such as TGF- $\beta$  (mean, -21.4 vs. -6.6 ng/mL;  $P<0.05$ ), pro-collagen III N-terminal peptide (mean, -10.4 vs. -4.9 ng/mL;  $P<0.05$ ), and tissue inhibitor of metalloproteinase 1 (mean, -169.6 vs. -66.2 ng/mL;  $P<0.001$ ). Marotta et al.<sup>246</sup> showed that vitamin E treatment in HCV-related cirrhosis patients improved parameters of the redox status, such as glutathione, glutathione disulfide (GSSG), glutathione/GSSG, and malondialdehyde (all  $P<0.005$ ). However, other studies failed to reveal an improvement in serum ALT level<sup>247-249</sup> or viral load.<sup>248</sup> Furthermore, cessation of vitamin E treatment was followed by a rapid relapse of ALT elevation and viremia.<sup>244,250</sup>

Most of the studies on the effects of vitamin E in HCV patients were conducted during a period in which IFN- $\alpha$  treatment was the standard treatment for HCV, and their results are also inconsistent. In recent years, as DAAs, which are very effective treatments, have become the standard therapeutic approach for HCV, further studies are needed on the role of vitamin E treatment in HCV patients in the DAA era.

Studies on the effectiveness of vitamin E treatment in HBV patients are very rare. Andreone et al.<sup>251</sup> reported that ALT normalization (47% vs. 6%;  $P=0.011$ ) and a complete response (47% vs. 0%;  $P=0.0019$ ) were achieved at a significantly higher rate following vitamin E treatment (600 IU/day) for 3 months compared to the control group among patients with a positive HBV DNA status and heightened ALT levels.

Vitamin E treatment decreased the serum aminotransferase level and improved inflammatory cytokine, chemokine, and adipokine concentrations in NAFLD patients with its antioxidant and anti-inflammatory properties. In addition, vitamin E treatment not only led to histological improvement in hepatic steatosis, lobular inflammation, and ballooning but also showed a potential to improve hepatic fibrosis in histologically proven NASH patients. Therefore, vitamin E may be an alternative treatment option for NASH patients who have no effective treatment other than weight loss. On the other

side, the usefulness of vitamin E has been reduced due to the use of effective antiviral agents in chronic viral hepatitis, and further studies are necessary on the role of vitamin E treatment.

### Safety

There are concerns about the long-term safety of vitamin E treatment. Some studies reported that vitamin E treatment is associated with an increase in overall mortality,<sup>252,253</sup> prostate cancer,<sup>254</sup> and hemorrhagic stroke.<sup>255</sup> In a meta-analysis of preventive studies of antioxidant supplements, vitamin E (duration, 0.5–6.3 years; dose, 16.5–800 IU/day) was significantly associated with increased mortality (RR, 1.04; 95% CI, 1.01–1.07).<sup>253</sup> In another meta-analysis of trials, high-dose vitamin E ( $\geq 400$  IU/day) for  $> 1$  year led to an increased risk for all-cause mortality ( $P=0.022$ ), and there was a significant relationship between vitamin E dosage and all-cause mortality ( $P=0.027$ ).<sup>252</sup> In a prospective study comparing the long-term effect of selenium (200 µg/day), vitamin E (400 IU/day), selenium plus vitamin E, and placebo, vitamin E treatment (follow-up period, 7–12 years) increased the risk of prostate cancer compared to placebo (HR, 1.17; 99% CI, 1.004–1.36;  $P=0.008$ ).<sup>254</sup> A meta-analysis of RCTs with  $\geq 1$  year of follow-up investigating the effect of vitamin E (50–800 IU/day) on stroke showed that vitamin E increased the risk for hemorrhagic stroke (RR, 1.22; 95% CI, 1.00–1.48;  $P=0.048$ ).<sup>255</sup> Therefore, long-term use of vitamin E should be avoided.

## ASPIRIN

### Mechanism of action

Following tissue injury, platelet activation and degranulation mediate the normal physiological tissue repair process. However, in chronic inflammation, over-activation of the platelet can cause fibrosis in various tissues.<sup>256</sup> In the liver, platelets could directly activate HSCs<sup>257</sup> or could interact with many effector cells, such as macrophages, cytotoxic T-cells, and natural killer T (NKT) cells, eventually causing hepatic fibrosis.<sup>258</sup> Aspirin could prevent hepatic fibrosis progression and HCC directly by blocking platelet function (Fig. 1).

Extrahepatic platelet-derived growth factor β produced by platelets may have directly activated HSCs in a biliary fibrosis model.<sup>257</sup> Platelet number and activation were increased in a

NASH model. In the same study, a platelet receptor subunit, glycoprotein 1b alpha (GP1ba), was an important mediator in NASH. The combination of GP1ba, hyaluronic acids, cytokines, and chemokines mediates the immune response, causing progression to NASH and HCC.<sup>259</sup> The crosstalk between cytotoxic T-cells, NKT cells, and hepatocytes can cause liver disease progression. Hepatocyte-driven lymphotoxin β receptor and NF-κB signaling could trigger NASH to HCC progression.<sup>258</sup> Prolyl 4-hydroxylase subunit α2 inhibition by aspirin could also decrease collagen deposition and HCC development.<sup>260</sup>

In a viral hepatitis model, platelets mediated cytotoxic T-cell-mediated liver damage. Aspirin treatment reduced the platelet activation, and intrahepatic cytokine release related in the inflammation process.<sup>27</sup> Aspirin treatment also reduces the homing and accumulation of virus specific cytotoxic T-cells as well as virus-non-specific lymphocytes in the liver.<sup>261</sup>

### Clinically beneficial effects

#### NAFLD

A nationwide cross-sectional study in the United States showed that regular aspirin use was associated with a lower prevalence of NAFLD itself (HR, 0.62; 95% CI, 0.51–0.74,  $P=0.04$ ) (Table 8).<sup>262</sup> A prospective cohort study with biopsy-confirmed NAFLD patients showed that aspirin use reduced the risk of advanced fibrosis (HR, 0.63; 95% CI, 0.43–0.85).<sup>263</sup> Another cross-sectional study using nationwide health survey data from the United States showed that regular aspirin use was associated with a decreased risk of liver fibrosis in CLD patients confirmed by ultrasonography ( $\beta$ -coefficients measured at 0.24 standard deviations lower; 95% CI, -0.42 to -0.06;  $P=0.009$ ).<sup>256</sup> To date, two studies have shown the association between aspirin use and the risk of HCC development. In a pooled analysis of 10 prospective cohort studies in the United States, aspirin led to a 32% decrease in HCC development (HR, 0.68; 95% CI, 0.57–0.81).<sup>264</sup> Meanwhile, in another pooled analysis of two prospective cohort studies in the United States, aspirin caused a 46% decrease in HCC development (HR, 0.54; 95% CI, 0.36–0.80).<sup>265</sup> Although both pooled analysis studies did not specify the etiology of liver disease, it is assumed that NAFLD may be the major etiology of liver disease.

In NAFLD patients, aspirin use was associated with various outcomes, such as prevalence, fibrosis, and HCC develop-

**Table 8.** Summary of clinical studies in aspirin

Study	Etiology	Inclusion criteria	Intervention period	Arms (n)	Age (years)	Outcomes	Study design
Simon et al. <sup>265</sup> (2018)	Variable	Pooled analysis of cohort	NA	Aspirin (58,855) Control (74,516)	64±8 62±8	· Decreased HCC development in aspirin group (HR, 0.54; 95% CI, 0.36–0.80)	Prospective cohort study
Petrick et al. <sup>264</sup> (2015)	Variable	Pooled analysis of cohort	NA	Aspirin (477,470) Control (606,663)	NA NA	· Decreased HCC development in aspirin group (HR, 0.68; 95% CI, 0.57–0.81)	Prospective cohort study
Simon et al. <sup>263</sup> (2019)	NAFLD	Biopsy confirmed NAFLD	NA	Aspirin (151) Control (210)	59.9±8.6 48.2±13.5	· Decreased prevalence of advanced fibrosis in aspirin group (HR, 0.63; 95% CI, 0.43–0.85)	Prospective cohort study
Shen et al. <sup>262</sup> (2014)	NAFLD	US-confirmed NAFLD	1 month	NAFLD (2,889) Control (8,527)	54.6±0.3 48.7±0.5	· Decreased NAFLD prevalence in aspirin group (HR, 0.62; 95% CI, 0.51–0.74)	Cross-sectional retrospective study
Jiang et al. <sup>256</sup> (2016)	CLD	US-confirmed CLD	1 month	Aspirin (520) Control (1,336)	46.6±15.4 43.2±14.7	· Decreased non-invasive fibrosis index in aspirin group, 0.24 standard deviation lower; 95% CI, -0.42 to 0.06	Cross-sectional retrospective study
Hui et al. <sup>271</sup> (2021)	CHB	Receiving nucleos(t)ide analog	Over 90 days	Aspirin (1,744) Control (33,367)	62.2±10.8 52.5±12.5	· Decreased HCC development in aspirin group (HR, 0.60; 95% CI, 0.46–0.78)	Retrospective cohort study
Choi et al. <sup>267</sup> (2021)	CHB		Over 90 days	Aspirin (7,718) Control (24,977)	NA NA	· Decreased HCC development in aspirin group (OR, 0.92; 95% CI, 0.85–0.99)	Retrospective cohort study
Simon et al. <sup>15</sup> (2020)	Viral hepatitis	CHB, CHC monoinfection	Over 90 days	Aspirin (14,205) Control (36,070)	50.5±13.0 39.6±13.5	· Decreased HCC development in aspirin group (HR, 0.69; 95% CI, 0.62–0.76) · Decreased liver-related death in aspirin group (HR, 0.73; 95% CI, 0.67–0.81)	Prospective cohort study
Liao et al. <sup>270</sup> (2020)	CHC		NA	Aspirin (1,991) Control (1,991)	NA NA	· Decreased HCC development in aspirin group (HR, 0.56; 95% CI, 0.43–0.72)	Retrospective cohort study
Lee et al. <sup>268</sup> (2020)	CHC		Over 90 days	Aspirin (2,478) Control (4,956)	63.2±10.0 63.2±10.0	· Decreased HCC development in aspirin group (HR, 0.78; 95% CI, 0.64–0.95)	Retrospective cohort study
Lee et al. <sup>269</sup> (2019)	CHB		Over 90 days	Aspirin (2,123) Control (8,492)	58.9±11.8 58.8±11.8	· Decreased HCC development in aspirin group (HR, 0.71; 95% CI, 0.58–0.86)	Retrospective cohort study
Lee et al. <sup>276</sup> (2017)	CHB	Low-level viremia	Median 38.5 months	Aspirin (343) Control (1,116)	54.2±11.1 50.3±10.8	· Decreased HCC development in aspirin group (HR, 0.26; 95% CI, 0.09–0.74)	Retrospective cohort study

Variables are expressed as median (interquartile range) or mean±standard deviation.  
NA, not applicable; HCC, hepatocellular carcinoma; US, ultrasonography; CLD, chronic liver disease;  
CHB, chronic hepatitis B; OR, odds ratio; CHC, chronic hepatitis C.

ment. However, there are still very few studies considering the outcomes of aspirin use in NAFLD. Since it is difficult to perform randomized controlled studies with aspirin, there is an unmet need for more cohort studies involving NAFLD patients.

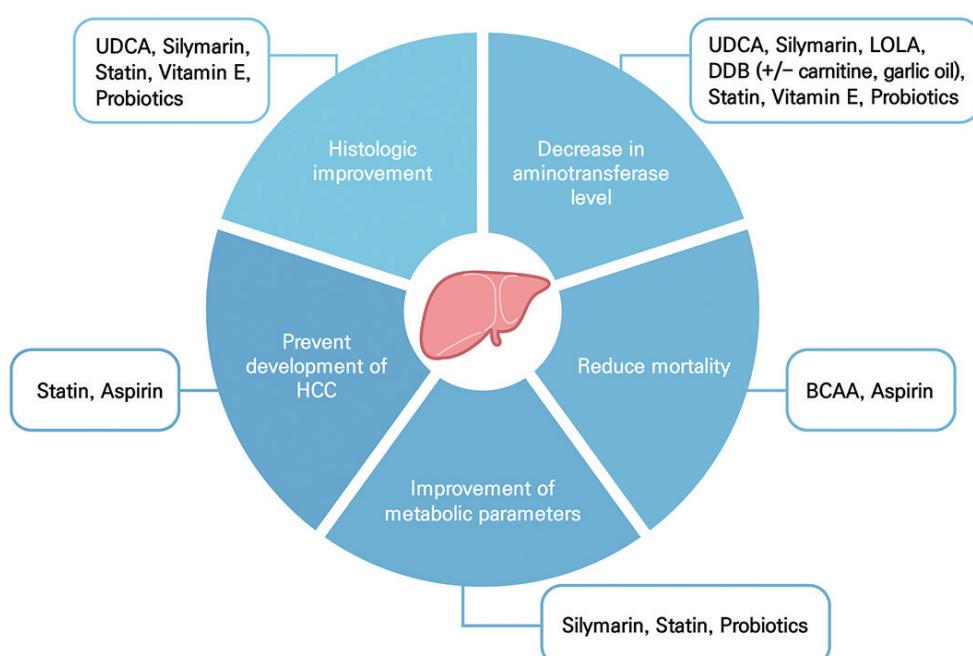
### Viral hepatitis

Most studies performed in viral hepatitis analyzed HCC development as primary outcome (Table 8). A recent meta-analysis of seven studies that studied CHB or CHC patients showed a 27% decrease in HCC development.<sup>266</sup> A prospective study using Swedish nationwide registry data including CHB or CHC patients with median 7.9 years of follow-up revealed a 31% decrease in HCC development in the aspirin group (HR, 0.69; 95% CI, 0.62–0.76). The reduction in HCC development was duration-dependent.<sup>15</sup> The authors also evaluated survival data and showed a 27% reduction in liver-related death in the aspirin treatment group (HR, 0.73; 95% CI, 0.67–0.81).<sup>15</sup> In addition, several population-based studies have been made available, mostly enrolling the Asian population. A Korean study using nationwide reimbursement data showed an 8% decrease in HCC development (OR, 0.92; 95% CI, 0.85–0.99).<sup>267</sup> Two Taiwan studies using nationwide reim-

bbursement data revealed a 29% decrease (HR, 0.71; 95% CI, 0.58–0.86;  $P<0.001$ ) and a 22% decrease (HR, 0.78; 95% CI, 0.64–0.95;  $P=0.011$ ), respectively, in HCC development in CHB and CHC patients.<sup>268,269</sup> Another Taiwan study using nationwide reimbursement data compared aspirin using CHC patients matched 1:1 with aspirin non-using CHC patients. The result included a 44% rate of HCC reduction (HR, 0.56; 95% CI, 0.43–0.72;  $P<0.001$ ).<sup>270</sup> A Hong Kong study using a nationwide electronic healthcare data repository analyzed antiviral-treated CHB patients and showed a 40% decrease in HCC development (HR, 0.60; 95% CI, 0.46–0.78,  $P<0.001$ ).<sup>271</sup>

In addition, several studies have shown the beneficial effects of aspirin use in prolonging OS in HCC patients treated with transarterial chemoembolization and curative resection.<sup>272,273</sup> In addition, it has been revealed that aspirin use can reduce HCC recurrence in several clinical settings.<sup>272,274,275</sup>

Collectively, in patients with viral hepatitis, aspirin seems to reduce the risk of HCC development. However, few studies have adopted liver fibrosis or OS as their primary endpoints, and most studies have enrolled population-based cohorts. Accordingly, further prospective studies based on hospital data with varying endpoints are warranted for solid validation.



**Figure 3.** Clinically beneficial effects of non-antidiabetic drugs in chronic liver diseases. UDCA, ursodeoxycholic acid; LOLA, L-ornithine L-aspartate; DDB, dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxypiphenyl-2,2'-dicarboxylate; HCC, hepatocellular carcinoma.

### Safety

The safety concern related to aspirin therapy is mainly gastrointestinal bleeding. Most studies did not report an increased risk of gastrointestinal bleeding in the aspirin group.<sup>15,269,276</sup> However, one study reported increased peptic ulcer bleeding in cirrhosis patients with aspirin use compared to aspirin-untreated patients.<sup>268</sup> Another study reported increased gastrointestinal bleeding in patients only with short-term aspirin use.<sup>271</sup>

## CONCLUSION

In this review, we summarized the mechanisms of several non-antidiabetics and their evidence regarding a beneficial effect in patients with CLD. Although the evidence is not sufficiently solid, the different nonanti-diabetics showed beneficial effects in improving histology, aminotransferase level, metabolic parameters, and the risk of long-term outcomes in patients with CLD without significant safety concerns (Fig. 3). However, further studies are still warranted to consolidate their potential benefit in adjuvant or combination settings in the era of potent antiviral therapy.

### Authors' contribution

Conception and design: S.U. Kim and Y.J. Kim; Writing, review, and/or revision of the manuscript: H.A. Lee, Y. Chang, P.S. Sung, E.L. Yoon, H.W. Lee, J. Yoo, Y. Lee, J. An, D.S. Song, Y.Y. Cho, S.U. Kim, and Y.J. Kim.

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### Conflicts of Interest

Seung Up Kim has served as an advisory committee member for Gilead Sciences, GSK, Bayer, Novo Nordisk, and Eisai. He is a speaker for Gilead Sciences, GSK, Bayer, Eisai, Abbvie, EchoSens, MSD, Bristol-Myers Squibb, Hanhwa, Yuhan, Samil, PharmaKing, Celltrion, and Bukwang. He has also received research grants from Abbvie and Bristol-Myers Squibb.

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Other authors have no conflicts to disclose.

## REFERENCES

1. Moon AM, Singal AG, Tapper EB. Contemporary epidemiology of chronic liver disease and cirrhosis. *Clin Gastroenterol Hepatol* 2020;18:2650-2666.
2. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018;392:1789-1858.
3. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006;44:217-231.
4. Kang SH, Lee HW, Yoo JJ, Cho Y, Kim SU, Lee TH, et al. KASL clinical practice guidelines: management of nonalcoholic fatty liver disease. *Clin Mol Hepatol* 2021;27:363-401.
5. Korean Association for the Study of the Liver (KASL). KASL clinical practice guidelines for management of chronic hepatitis B. *Clin Mol Hepatol* 2019;25:93-159.
6. Mbaga DS, Kenmoe S, Kengne-Ndé C, Ebogo-Belobo JT, Mahamat G, Foe-Essomba JR, et al. Hepatitis B, C and D virus infections and risk of hepatocellular carcinoma in Africa: a meta-analysis including sensitivity analyses for studies comparable

- for confounders. *PLoS One* 2022;17:e0262903.
7. Lee YB, Ha Y, Chon YE, Kim MN, Lee JH, Park H, et al. Association between hepatic steatosis and the development of hepatocellular carcinoma in patients with chronic hepatitis B. *Clin Mol Hepatol* 2019;25:52-64.
  8. Lindor KD, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, et al. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. *Hepatology* 2004;39:770-778.
  9. Navarro VJ, Belle SH, D'Amato M, Adfhal N, Brunt EM, Fried MW, et al. Silymarin in non-cirrhotics with non-alcoholic steatohepatitis: a randomized, double-blind, placebo controlled trial. *PLoS One* 2019;14:e0221683.
  10. Lee SH, Cheon GJ, Kim HS, Kim YD, Kim SG, Kim YS, et al. Comparison on the efficacy and safety of biphenyl dimethyl dicarboxylate and ursodeoxycholic acid in patients with abnormal alanine aminotransferase: multicenter, double-blinded, randomized, active-controlled clinical trial. *Korean J Gastroenterol* 2014;64:31-39.
  11. Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, et al. Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. *Clin Gastroenterol Hepatol* 2005;3:705-713.
  12. Nelson A, Torres DM, Morgan AE, Fincke C, Harrison SA. A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: a randomized placebo-controlled trial. *J Clin Gastroenterol* 2009;43:990-994.
  13. Eslamiparast T, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hekmatdoost A. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Am J Clin Nutr* 2014;99:535-542.
  14. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362:1675-1685.
  15. Simon TG, Duberg AS, Aleman S, Chung RT, Chan AT, Ludvigsson JF. Association of aspirin with hepatocellular carcinoma and liver-related mortality. *N Engl J Med* 2020;382:1018-1028.
  16. Poupon R. Ursodeoxycholic acid and bile-acid mimetics as therapeutic agents for cholestatic liver diseases: an overview of their mechanisms of action. *Clin Res Hepatol Gastroenterol* 2012;36 Suppl 1:S3-S12.
  17. Roma MG, Toledo FD, Boaglio AC, Basiglio CL, Crocenzi FA, Sánchez Pozzi EJ. Ursodeoxycholic acid in cholestasis: linking action mechanisms to therapeutic applications. *Clin Sci (Lond)* 2011;121:523-544.
  18. Surai PF. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. *Antioxidants (Basel)* 2015;4:204-247.
  19. Fu T, Liu G. Protective effects of dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate on damages of isolated rat hepatocytes induced by carbon tetrachloride and D-galactosamine. *Biomed Environ Sci* 1992;5:185-194.
  20. De Chiara F, Heebøll S, Marrone G, Montoliu C, Hamilton-Dutoit S, Ferrandez A, et al. Urea cycle dysregulation in non-alcoholic fatty liver disease. *J Hepatol* 2018;69:905-915.
  21. Thomsen KL, De Chiara F, Rombouts K, Vilstrup H, Andreola F, Mookerjee RP, et al. Ammonia: a novel target for the treatment of non-alcoholic steatohepatitis. *Med Hypotheses* 2018;113:91-97.
  22. Kimball SR, Jefferson LS. New functions for amino acids: effects on gene transcription and translation. *Am J Clin Nutr* 2006;83:500S-507S.
  23. Chou R, Dana T, Blazina I, Daeges M, Jeanne TL. Statins for prevention of cardiovascular disease in adults: evidence report and systematic review for the US preventive services task force. *JAMA* 2016;316:2008-2024.
  24. Jain A, Sharma BC, Mahajan B, Srivastava S, Kumar A, Sachdeva S, et al. L-ornithine L-aspartate in acute treatment of severe hepatic encephalopathy: a double-blind randomized controlled trial. *Hepatology* 2022;75:1194-1203.
  25. Porras D, Nistal E, Martínez-Flórez S, González-Gallego J, García-Mediavilla MV, Sánchez-Campos S. Intestinal microbiota modulation in obesity-related non-alcoholic fatty liver disease. *Front Physiol* 2018;9:1813.
  26. Zhang Y, Hai J, Cao M, Zhang Y, Pei S, Wang J, et al. Silibinin ameliorates steatosis and insulin resistance during non-alcoholic fatty liver disease development partly through targeting IRS-1/PI3K/Akt pathway. *Int Immunopharmacol* 2013;17:714-720.
  27. Iannaccone M, Sitia G, Isogawa M, Marchese P, Castro MG, Lowenstein PR, et al. Platelets mediate cytotoxic T lymphocyte-induced liver damage. *Nat Med* 2005;11:1167-1169.
  28. Hajighamhamadi AA, Ziae A, Oveis S, Masroor H. Effects of metformin, pioglitazone, and silymarin treatment on non-alcoholic fatty liver disease: a randomized controlled pilot study. *Hepat Mon* 2012;12:e6099.
  29. Takeshita Y, Takamura T, Kita Y, Ando H, Ueda T, Kato K, et al. Beneficial effect of branched-chain amino acid supplementation on glycemic control in chronic hepatitis C patients with in-

- sulin resistance: implications for type 2 diabetes. *Metabolism* 2012;61:1388-1394.
- 30. Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, et al. *Bifidobacterium longum* with fructo-oligo-saccharides in patients with non alcoholic steatohepatitis. *Dig Dis Sci* 2012;57:545-553.
  - 31. Wah Kheong C, Nik Mustapha NR, Mahadeva S. A randomized trial of silymarin for the treatment of nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2017;15:1940-1949.e8.
  - 32. Les I, Doval E, García-Martínez R, Planas M, Cárdenas G, Gómez P, et al. Effects of branched-chain amino acids supplementation in patients with cirrhosis and a previous episode of hepatic encephalopathy: a randomized study. *Am J Gastroenterol* 2011;106:1081-1088.
  - 33. Scorletti E, Afolabi PR, Miles EA, Smith DE, Almehmadi A, Alshathry A, et al. Synbiotics alter fecal microbiomes, but not liver fat or fibrosis, in a randomized trial of patients with non-alcoholic fatty liver disease. *Gastroenterology* 2020;158:1597-1610.e7.
  - 34. Parikh P, Ingle M, Patel J, Bhate P, Pandey V, Sawant P. An open-label randomized control study to compare the efficacy of vitamin E versus ursodeoxycholic acid in nondiabetic and noncirrhotic Indian NAFLD patients. *Saudi J Gastroenterol* 2016;22:192-197.
  - 35. Lindor KD, Bowlus CL, Boyer J, Levy C, Mayo M. Primary biliary cholangitis: 2018 practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2019;69:394-419.
  - 36. Leuschner U, Fischer H, Kurtz W, Güldütuna S, Hübner K, Hellstern A, et al. Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. *Gastroenterology* 1989;97:1268-1274.
  - 37. Ikebuchi Y, Shimizu H, Ito K, Yoshikado T, Yamanashi Y, Takada T, et al. Ursodeoxycholic acid stimulates the formation of the bile canalicular network. *Biochem Pharmacol* 2012;84:925-935.
  - 38. Okada K, Shoda J, Taguchi K, Maher JM, Ishizaki K, Inoue Y, et al. Ursodeoxycholic acid stimulates Nrf2-mediated hepatocellular transport, detoxification, and antioxidative stress systems in mice. *Am J Physiol Gastrointest Liver Physiol* 2008;295:G735-G747.
  - 39. El-Sherbiny GA, Taye A, Abdel-Raheem IT. Role of ursodeoxycholic acid in prevention of hepatotoxicity caused by amoxicillin-clavulanic acid in rats. *Ann Hepatol* 2009;8:134-140.
  - 40. Yokoyama K, Tatsumi Y, Hayashi K, Goto H, Ishikawa T, Wakusawa S. Effects of ursodeoxycholic acid and insulin on palmitate-induced ROS production and down-regulation of PI3K/Akt signaling activity. *Biol Pharm Bull* 2017;40:2001-2004.
  - 41. Mueller M, Castro RE, Thorell A, Marschall HU, Auer N, Herac M, et al. Ursodeoxycholic acid: effects on hepatic unfolded protein response, apoptosis and oxidative stress in morbidly obese patients. *Liver Int* 2018;38:523-531.
  - 42. Amaral JD, Viana RJ, Ramalho RM, Steer CJ, Rodrigues CM. Bile acids: regulation of apoptosis by ursodeoxycholic acid. *J Lipid Res* 2009;50:1721-1734.
  - 43. Solá S, Castro RE, Kren BT, Steer CJ, Rodrigues CM. Modulation of nuclear steroid receptors by ursodeoxycholic acid inhibits TGF-beta1-induced E2F-1/p53-mediated apoptosis of rat hepatocytes. *Biochemistry* 2004;43:8429-8438.
  - 44. Castro RE, Ferreira DM, Afonso MB, Borralho PM, Machado MV, Cortez-Pinto H, et al. miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. *J Hepatol* 2013;58:119-125.
  - 45. Mueller M, Thorell A, Claudel T, Jha P, Koefeler H, Lackner C, et al. Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity. *J Hepatol* 2015;62:1398-1404.
  - 46. Wu P, Zhao J, Guo Y, Yu Y, Wu X, Xiao H. Ursodeoxycholic acid alleviates nonalcoholic fatty liver disease by inhibiting apoptosis and improving autophagy via activating AMPK. *Biochem Biophys Res Commun* 2020;529:834-838.
  - 47. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. *Hepatology* 1990;11:12-15.
  - 48. Oh AR, Bae JS, Lee J, Shin E, Oh BC, Park SC, et al. Ursodeoxycholic acid decreases age-related adiposity and inflammation in mice. *BMB Rep* 2016;49:105-110.
  - 49. Miura T, Ouchida R, Yoshikawa N, Okamoto K, Makino Y, Nakamura T, et al. Functional modulation of the glucocorticoid receptor and suppression of NF-kappaB-dependent transcription by ursodeoxycholic acid. *J Biol Chem* 2001;276:47371-47378.
  - 50. Takigawa T, Miyazaki H, Kinoshita M, Kawarabayashi N, Nishiyama K, Hatsuse K, et al. Glucocorticoid receptor-dependent immunomodulatory effect of ursodeoxycholic acid on liver lymphocytes in mice. *Am J Physiol Gastrointest Liver Physiol* 2013;305:G427-G438.
  - 51. Shimoyama S, Kawata K, Ohta K, Chida T, Suzuki T, Tsuneyama K, et al. Ursodeoxycholic acid impairs liver-infiltrating T-cell

- chemotaxis through IFN- $\gamma$  and CX3CL1 production in primary biliary cholangitis. *Eur J Immunol* 2021;51:1519-1530.
52. Lee EJ, Kwon JE, Park MJ, Jung KA, Kim DS, Kim EK, et al. Ursodeoxycholic acid attenuates experimental autoimmune arthritis by targeting Th17 and inducing pAMPK and transcriptional corepressor SMILE. *Immunol Lett* 2017;188:1-8.
53. Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, et al. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. *Hepatology* 1996;23:1464-1467.
54. Dufour JF, Oneta CM, Gonvers JJ, Bihl F, Cerny A, Cereda JM, et al. Randomized placebo-controlled trial of ursodeoxycholic acid with vitamin E in nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2006;4:1537-1543.
55. Balmer ML, Siegrist K, Zimmermann A, Dufour JF. Effects of ursodeoxycholic acid in combination with vitamin E on adiponectines and apoptosis in patients with nonalcoholic steatohepatitis. *Liver Int* 2009;29:1184-1188.
56. Colombo C, Crosignani A, Assaiso M, Battezzati PM, Podda M, Giunta A, et al. Ursodeoxycholic acid therapy in cystic fibrosis-associated liver disease: a dose-response study. *Hepatology* 1992;16:924-930.
57. Mitchell SA, Bansi DS, Hunt N, Von Bergmann K, Fleming KA, Chapman RW. A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. *Gastroenterology* 2001;121:900-907.
58. Leuschner UF, Lindenthal B, Herrmann G, Arnold JC, Rössle M, Cordes HJ, et al. High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. *Hepatology* 2010;52:472-479.
59. Ratziu V, de Ledinghen V, Oberti F, Mathurin P, Wartelle-Bladou C, Renou C, et al. A randomized controlled trial of high-dose ursodesoxycholic acid for nonalcoholic steatohepatitis. *J Hepatol* 2011;54:1011-1019.
60. Yoon YB, Hagey LR, Hofmann AF, Gurantz D, Michelotti EL, Steinbach JH. Effect of side-chain shortening on the physiologic properties of bile acids: hepatic transport and effect on biliary secretion of 23-nor-ursodeoxycholate in rodents. *Gastroenterology* 1986;90:837-852.
61. Steinacher D, Claudel T, Trauner M. Therapeutic mechanisms of bile acids and nor-ursodeoxycholic acid in non-alcoholic fatty liver disease. *Dig Dis* 2017;35:282-287.
62. Beraza N, Ofner-Ziegenfuss L, Ehedego H, Boekschoten M, Bischoff SC, Mueller M, et al. Nor-ursodeoxycholic acid reverses hepatocyte-specific nemo-dependent steatohepatitis. *Gut* 2011;60:387-396.
63. Traussnigg S, Schattenberg JM, Demir M, Wiegand J, Geier A, Teuber G, et al. Norursodeoxycholic acid versus placebo in the treatment of non-alcoholic fatty liver disease: a double-blind, randomised, placebo-controlled, phase 2 dose-finding trial. *Lancet Gastroenterol Hepatol* 2019;4:781-793.
64. Fabbri C, Marchetto S, Pezzoli A, Accogli E, Fusaroli P, Azzaroli F, et al. Efficacy of ursodeoxycholic acid in association with alpha-interferon for chronic hepatitis C in alpha-interferon non-responder patients. *Eur J Gastroenterol Hepatol* 2000;12:511-515.
65. Boucher E, Guyader D, Jacquelin S, Andre P, Mendler MH, Turlin B, et al. Interferon and ursodeoxycholic acid combined therapy in chronic viral C hepatitis: controlled randomized trial in 203 patients. *Dig Liver Dis* 2000;32:29-33.
66. Omata M, Yoshida H, Toyota J, Tomita E, Nishiguchi S, Hayashi N, et al. A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. *Gut* 2007;56:1747-1753.
67. European Association for the Study of the Liver. EASL clinical practice guidelines: the diagnosis and management of patients with primary biliary cholangitis. *J Hepatol* 2017;67:145-172.
68. Dehmlow C, Erhard J, de Groot H. Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin. *Hepatology* 1996;23:749-754.
69. Kwon DY, Jung YS, Kim SJ, Kim YS, Choi DW, Kim YC. Alterations in sulfur amino acid metabolism in mice treated with silymarin: a novel mechanism of its action involved in enhancement of the antioxidant defense in liver. *Planta Med* 2013;79:997-1002.
70. Song Z, Deaciuc I, Song M, Lee DY, Liu Y, Ji X, et al. Silymarin protects against acute ethanol-induced hepatotoxicity in mice. *Alcohol Clin Exp Res* 2006;30:407-413.
71. Valenzuela A, Garrido A. Biochemical bases of the pharmacological action of the flavonoid silymarin and of its structural isomer silibinin. *Biol Res* 1994;27:105-112.
72. Valenzuela A, Guerra R. Differential effect of silybin on the Fe<sup>2+</sup>-ADP and t-butyl hydroperoxide-induced microsomal lipid peroxidation. *Experientia* 1986;42:139-141.
73. van Pelt JF, Verslype C, Crabbé T, Zaman Z, Fevery J. Primary human hepatocytes are protected against prolonged and repeated exposure to ethanol by silibinin-dihemisuccinate. *Alcohol Alcohol* 2003;38:411-414.
74. Dehmlow C, Murawski N, de Groot H. Scavenging of reactive

- oxygen species and inhibition of arachidonic acid metabolism by silibinin in human cells. *Life Sci* 1996;58:1591-1600.
75. Valenzuela A, Guerra R, Garrido A. Silybin dihemisuccinate protects rat erythrocytes against phenylhydrazine-induced lipid peroxidation and hemolysis. *Planta Med* 1987;53:402-405.
76. Noel-Hudson MS, de Belilovsky C, Petit N, Lindenbaum A, Wepierre J. In vitro cytotoxic effects of enzymatically induced oxygen radicals in human fibroblasts: experimental procedures and protection by radical scavengers. *Toxicol In Vitro* 1989;3:103-109.
77. Salomone F, Barbagallo I, Godos J, Lembo V, Currenti W, Cinà D, et al. Silibinin restores NAD<sup>+</sup> levels and induces the SIRT1/AMPK pathway in non-alcoholic fatty liver. *Nutrients* 2017;9:1086.
78. Hahn G, Lehmann HD, Kürten M, Uebel H, Vogel G. On the pharmacology and toxicology of silymarin, an antihepatotoxic active principle from *Silybum marianum* (L.) Gaertn. *Arzneimittelforschung* 1968;18:698-704.
79. Lieber CS, Leo MA, Cao Q, Ren C, DeCarli LM. Silymarin retards the progression of alcohol-induced hepatic fibrosis in baboons. *J Clin Gastroenterol* 2003;37:336-339.
80. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.
81. Trappoliere M, Caligiuri A, Schmid M, Bertolani C, Failli P, Vizzutti F, et al. Silybin, a component of sylimarin, exerts anti-inflammatory and anti-fibrogenic effects on human hepatic stellate cells. *J Hepatol* 2009;50:1102-1111.
82. Kim M, Yang SG, Kim JM, Lee JW, Kim YS, Lee JI. Silymarin suppresses hepatic stellate cell activation in a dietary rat model of non-alcoholic steatohepatitis: analysis of isolated hepatic stellate cells. *Int J Mol Med* 2012;30:473-479.
83. Yao J, Zhi M, Gao X, Hu P, Li C, Yang X. Effect and the probable mechanisms of silibinin in regulating insulin resistance in the liver of rats with non-alcoholic fatty liver. *Braz J Med Biol Res* 2013;46:270-277.
84. Gu M, Zhao P, Huang J, Zhao Y, Wang Y, Li Y, et al. Silymarin ameliorates metabolic dysfunction associated with diet-induced obesity via activation of farnesyl X receptor. *Front Pharmacol* 2016;7:345.
85. Solhi H, Ghahremani R, Kazemifar AM, Hoseini Yazdi Z. Silymarin in treatment of non-alcoholic steatohepatitis: a randomized clinical trial. *Caspian J Intern Med* 2014;5:9-12.
86. Hashemi SJ, Hajiani E, Heydari SE. A placebo-controlled trial of silymarin in patients with nonalcoholic fatty liver disease. *Hepat Mon* 2009;9:265-270.
87. Anushiravani A, Haddadi N, Pourfarmanbar M, Mohammad-karimi V. Treatment options for nonalcoholic fatty liver disease: a double-blinded randomized placebo-controlled trial. *Eur J Gastroenterol Hepatol* 2019;31:613-617.
88. Stiuso P, Scognamiglio I, Murolo M, Ferranti P, De Simone C, Rizzo MR, et al. Serum oxidative stress markers and lipidomic profile to detect NASH patients responsive to an antioxidant treatment: a pilot study. *Oxid Med Cell Longev* 2014;2014:169216.
89. Kalopitas G, Antza C, Doundoulakis I, Siargkas A, Kouroumalis E, Germanidis G, et al. Impact of silymarin in individuals with nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Nutrition* 2021;83:111092.
90. Sorrentino G, Crispino P, Coppola D, De Stefano G. Efficacy of lifestyle changes in subjects with non-alcoholic liver steatosis and metabolic syndrome may be improved with an antioxidant nutraceutical: a controlled clinical study. *Drugs R D* 2015;15:21-25.
91. Fried MW, Navarro VJ, Afdhal N, Belle SH, Wahed AS, Hawke RL, et al. Effect of silymarin (milk thistle) on liver disease in patients with chronic hepatitis C unsuccessfully treated with interferon therapy: a randomized controlled trial. *JAMA* 2012;308:274-282.
92. Yakoot M, Salem A. Spirulina platensis versus silymarin in the treatment of chronic hepatitis C virus infection. A pilot randomized, comparative clinical trial. *BMC Gastroenterol* 2012;12:32.
93. Kalantari H, Shahshahan Z, Hejazi SM, Ghafghazi T, Sebghato-lahi V. Effects of silybum marianum on patients with chronic hepatitis C. *J Res Med Sci* 2011;16:287-290.
94. Tanamly MD, Tadros F, Labeeb S, Makld H, Shehata M, Mikhail N, et al. Randomised double-blinded trial evaluating silymarin for chronic hepatitis C in an Egyptian village: study description and 12-month results. *Dig Liver Dis* 2004;36:752-759.
95. Ferenci P, Scherzer TM, Kerschner H, Rutter K, Beinhardt S, Hofer H, et al. Silibinin is a potent antiviral agent in patients with chronic hepatitis C not responding to pegylated interferon/ribavirin therapy. *Gastroenterology* 2008;135:1561-1567.
96. Saller R, Brignoli R, Melzer J, Meier R. An updated systematic review with meta-analysis for the clinical evidence of silymarin. *Fortsch Komplementmed* 2008;15:9-20.
97. Li XY. Bioactivity of neolignans from *fructus Schizandrae*. *Mem Inst Oswaldo Cruz* 1991;86 Suppl 2:31-37.
98. El-Beshbishi HA. The effect of dimethyl dimethoxy biphenyl

- dicarboxylate (DDB) against tamoxifen-induced liver injury in rats: DDB use is curative or protective. *J Biochem Mol Biol* 2005;38:300-306.
99. Kim SG, Kim HJ, Choi SH, Ryu JY. Inhibition of lipopolysaccharide-induced I-kappaB degradation and tumor necrosis factor-alpha expression by dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate (DDB): minor role in hepatic detoxifying enzyme expression. *Liver* 2000;20:319-329.
100. El-Bahy AA, Kassem LA, Heikal OA, Mahran LG. Antia apoptotic effect of DDB against hepatic ischemia-reperfusion injury. *J Toxicol Sci* 2011;36:145-154.
101. Rebouche CJ. Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. *Ann N Y Acad Sci* 2004;1033:30-41.
102. Jun DW, Cho WK, Jun JH, Kwon HJ, Jang KS, Kim HJ, et al. Prevention of free fatty acid-induced hepatic lipotoxicity by carnitine via reversal of mitochondrial dysfunction. *Liver Int* 2011;31:1315-1324.
103. Brady JF, Li DC, Ishizaki H, Yang CS. Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. *Cancer Res* 1988;48:5937-5940.
104. Hayes MA, Rushmore TH, Goldberg MT. Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydrazine by diallyl sulfide, a component of garlic oil. *Carcinogenesis* 1987;8:1155-1157.
105. Kim SG, Kwak JY, Lee JW, Novak RF, Park SS, Kim ND. Malotilate, a hepatoprotectant, suppresses CYP2E1 expression in rats. *Biochem Biophys Res Commun* 1994;200:1414-1420.
106. Kim SG, Nam SY, Chung HC, Hong SY, Jung KH. Enhanced effectiveness of dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate in combination with garlic oil against experimental hepatic injury in rats and mice. *J Pharm Pharmacol* 1995;47:678-682.
107. Park EY, Ki SH, Ko MS, Kim CW, Lee MH, Lee YS, et al. Garlic oil and DDB, comprised in a pharmaceutical composition for the treatment of patients with viral hepatitis, prevents acute liver injuries potentiated by glutathione deficiency in rats. *Chem Biol Interact* 2005;155:82-96.
108. Kang JS, Hong JH, Park MS, Lee MH, Lee JB, Lee JW. A randomized controlled, double-blind evaluation of the short-term(8 weeks) efficacy and safety of godex in Korean chronic hepatitis patients. *J Korean Soc Clin Pharmacol Ther* 2001;9:81-96.
109. Park MS, Kang JS, Chon CY, Paik SW, Rim KS, Kwak MJ, et al. Oral godex capsule for chronic liver disease: a double-blind, randomized, multicenter controlled trial. *J Korean Soc Clin Pharmacol Ther* 2001;9:151-162.
110. Kim HJ, Lee JS, Lee HW, Kim MY, Nam SW, Sohn JH, et al. The PERFECT Study (PEnnel Real liFe Efficacy Clinical Trial), a double-blind, randomized, multicenter trial examining the efficacy of biphenyl dimethyl dicarboxylate combined with garlic oil in patients with transaminase elevated chronic liver disease. *Korean J Med* 2014;86:179-189.
111. Hong ES, Kim EK, Kang SM, Khang AR, Choi SH, Park KS, et al. Effect of carnitine-orotate complex on glucose metabolism and fatty liver: a double-blind, placebo-controlled study. *J Gastroenterol Hepatol* 2014;29:1449-1457.
112. Bae JC, Lee WY, Yoon KH, Park JY, Son HS, Han KA, et al. Improvement of nonalcoholic fatty liver disease with carnitine-orotate complex in type 2 diabetes (CORONA): a randomized controlled trial. *Diabetes Care* 2015;38:1245-1252.
113. Jun DW, Kim BI, Cho YK, Kim HJ, Kwon YO, Park SY, et al. Efficacy and safety of entecavir plus carnitine complex (GODEX®) compared to entecavir monotherapy in patient with ALT elevated chronic hepatitis B: randomized, multicenter open-label trials. The GOAL study. *Clin Mol Hepatol* 2013;19:165-172.
114. Grüngreiff K, Baumann JL. Efficacy of L-ornithine L-aspartate granules in the treatment of chronic liver disease. *Med Welt* 2001;52:219-226.
115. Butterworth RF, Kircheis G, Hilger N, McPhail MJW. Efficacy of L-ornithine L-aspartate for the treatment of hepatic encephalopathy and hyperammonemia in cirrhosis: systematic review and meta-analysis of randomized controlled trials. *J Clin Exp Hepatol* 2018;8:301-313.
116. Gutiérrez-de-Juan V, López de Dávalillo S, Fernández-Ramos D, Barbier-Torres L, Zubiete-Franco I, Fernández-Tussy P, et al. A morphological method for ammonia detection in liver. *PLoS One* 2017;12:e0173914.
117. Jepsen P, Ott P, Andersen PK, Sørensen HT, Vilstrup H. Clinical course of alcoholic liver cirrhosis: a Danish population-based cohort study. *Hepatology* 2010;51:1675-1682.
118. Butterworth RF, Canbay A. Hepatoprotection by L-ornithine L-aspartate in non-alcoholic fatty liver disease. *Dig Dis* 2019;37:63-68.
119. Tian LY, Lu LG, Tang CW, Xie Y, Luo HS, Tan SY, et al. Aspartate-ornithine granules in the treatment of nonalcoholic steatohepatitis: a multiple-dose parallel controlled clinical trial. *Zhonghua Gan Zang Bing Za Zhi* 2013;21:528-532.
120. Ermolova T, Ermolov S. Correction of intrahepatic microcirculation disorders by L-ornithine-L-aspartate at the chronic liver

- diseases patients. *J Hepatol* 2018;68:S585-S586.
121. Rudler M, Weiss N, Bouzib C, Thabut D. Diagnosis and management of hepatic encephalopathy. *Clin Liver Dis* 2021;25:393-417.
122. Okuno M, Moriwaki H, Kato M, Muto Y, Kojima S. Changes in the ratio of branched-chain to aromatic amino acids affect the secretion of albumin in cultured rat hepatocytes. *Biochem Biophys Res Commun* 1995;214:1045-1050.
123. Ijichi C, Matsumura T, Tsuji T, Eto Y. Branched-chain amino acids promote albumin synthesis in rat primary hepatocytes through the mTOR signal transduction system. *Biochem Biophys Res Commun* 2003;303:59-64.
124. Montoya A, Gómez-Lechón MJ, Castell JV. Influence of branched-chain amino acid composition of culture media on the synthesis of plasma proteins by serum-free cultured rat hepatocytes. *In Vitro Cell Dev Biol* 1989;25:358-364.
125. Kuwahata M, Yoshimura T, Sawai Y, Amano S, Tomoe Y, Segawa H, et al. Localization of polypyrimidine-tract-binding protein is involved in the regulation of albumin synthesis by branched-chain amino acids in HepG2 cells. *J Nutr Biochem* 2008;19:438-447.
126. Nishitani S, Matsumura T, Fujitani S, Sonaka I, Miura Y, Yagasaki K. Leucine promotes glucose uptake in skeletal muscles of rats. *Biochem Biophys Res Commun* 2002;299:693-696.
127. Doi M, Yamaoka I, Fukunaga T, Nakayama M. Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. *Biochem Biophys Res Commun* 2003;312:1111-1117.
128. Nishitani S, Takehana K, Fujitani S, Sonaka I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. *Am J Physiol Gastrointest Liver Physiol* 2005;288:G1292-G1300.
129. Hinault C, Mothe-Satney I, Gautier N, Lawrence JC Jr, Van Obberghen E. Amino acids and leucine allow insulin activation of the PKB/mTOR pathway in normal adipocytes treated with wortmannin and in adipocytes from db/db mice. *FASEB J* 2004;18:1894-1896.
130. Higuchi N, Kato M, Miyazaki M, Tanaka M, Kohjima M, Ito T, et al. Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. *J Cell Biochem* 2011;112:30-38.
131. Iwao M, Gotoh K, Arakawa M, Endo M, Honda K, Seike M, et al. Supplementation of branched-chain amino acids decreases fat accumulation in the liver through intestinal microbiota-mediated production of acetic acid. *Sci Rep* 2020;10:18768.
132. Lee HL, Lee J, Cha JH, Cho S, Sung PS, Hur W, et al. Anti-fibrotic effects of branched-chain amino acids on hepatic stellate cells. *Korean J Intern Med* 2022;37:53-62.
133. Takegoshi K, Honda M, Okada H, Takabatake R, Matsuzawa-Nagata N, Campbell JS, et al. Branched-chain amino acids prevent hepatic fibrosis and development of hepatocellular carcinoma in a non-alcoholic steatohepatitis mouse model. *Oncotarget* 2017;8:18191-18205.
134. Khedr NF, Khedr EG. Branched chain amino acids supplementation modulates TGF- $\beta$ 1/Smad signaling pathway and interleukins in CCl4-induced liver fibrosis. *Fundam Clin Pharmacol* 2017;31:534-545.
135. Cha JH, Bae SH, Kim HL, Park NR, Choi ES, Jung ES, et al. Branched-chain amino acids ameliorate fibrosis and suppress tumor growth in a rat model of hepatocellular carcinoma with liver cirrhosis. *PLoS One* 2013;8:e77899.
136. Kakazu E, Kanno N, Ueno Y, Shimosegawa T. Extracellular branched-chain amino acids, especially valine, regulate maturation and function of monocyte-derived dendritic cells. *J Immunol* 2007;179:7137-7146.
137. Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, et al. Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res* 2006;35:204-214.
138. Kawamura E, Habu D, Morikawa H, Enomoto M, Kawabe J, Tamori A, et al. A randomized pilot trial of oral branched-chain amino acids in early cirrhosis: validation using prognostic markers for pre-liver transplant status. *Liver Transpl* 2009;15:790-797.
139. Nakaya Y, Okita K, Suzuki K, Moriwaki H, Kato A, Miwa Y, et al. BCAA-enriched snack improves nutritional state of cirrhosis. *Nutrition* 2007;23:113-120.
140. Koya S, Kawaguchi T, Hashida R, Goto E, Matsuse H, Saito H, et al. Effects of in-hospital exercise on liver function, physical ability, and muscle mass during treatment of hepatoma in patients with chronic liver disease. *Hepatol Res* 2017;47:E22-E34.
141. Habu D, Nishiguchi S, Nakatani S, Lee C, Enomoto M, Tamori A, et al. Comparison of the effect of BCAA granules on between decompensated and compensated cirrhosis. *Hepatogastroenterology* 2009;56:1719-1723.
142. Park JG, Tak WY, Park SY, Kweon YO, Chung WJ, Jang BK, et al. Effects of branched-chain amino acid (BCAA) supplementation on the progression of advanced liver disease: a Korean nation-

- wide, multicenter, prospective, observational, cohort study. *Nutrients* 2020;12:1429.
143. Park JG, Tak WY, Park SY, Kweon YO, Jang SY, Lee YR, et al. Effects of branched-chain amino acids (BCAAs) on the progression of advanced liver disease: a Korean nationwide, multicenter, retrospective, observational, cohort study. *Medicine (Baltimore)* 2017;96:e6580.
144. Marchesini G, Bianchi G, Merli M, Amodio P, Panella C, Loguerico C, et al. Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. *Gastroenterology* 2003;124:1792-1801.
145. Ichikawa T, Naota T, Miyaaki H, Miuma S, Isomoto H, Takeshima F, et al. Effect of an oral branched chain amino acid-enriched snack in cirrhotic patients with sleep disturbance. *Hepatol Res* 2010;40:971-978.
146. Ishikawa T. Early administration of branched-chain amino acid granules. *World J Gastroenterol* 2012;18:4486-4490.
147. Ismaiel A, Bucsa C, Farcas A, Leucuta DC, Popa SL, Dumitrascu DL. Effects of branched-chain amino acids on parameters evaluating sarcopenia in liver cirrhosis: systematic review and meta-analysis. *Front Nutr* 2022;9:749969.
148. Hanai T, Shiraki M, Imai K, Suetsugu A, Takai K, Shimizu M. Late evening snack with branched-chain amino acids supplementation improves survival in patients with cirrhosis. *J Clin Med* 2020;9:1013.
149. Koreeda C, Seki T, Okazaki K, Ha-Kawa SK, Sawada S. Effects of late evening snack including branched-chain amino acid on the function of hepatic parenchymal cells in patients with liver cirrhosis. *Hepatol Res* 2011;41:417-422.
150. Gluud LL, Dam G, Borre M, Les I, Cordoba J, Marchesini G, et al. Oral branched-chain amino acids have a beneficial effect on manifestations of hepatic encephalopathy in a systematic review with meta-analyses of randomized controlled trials. *J Nutr* 2013;143:1263-1268.
151. Gluud LL, Dam G, Les I, Marchesini G, Borre M, Aagaard NK, et al. Branched-chain amino acids for people with hepatic encephalopathy. *Cochrane Database Syst Rev* 2017;5:CD001939.
152. Dam G, Aamann L, Vistrup H, Gluud LL. The role of branched chain amino acids in the treatment of hepatic encephalopathy. *J Clin Exp Hepatol* 2018;8:448-451.
153. Dam G, Ott P, Aagaard NK, Vilstrup H. Branched-chain amino acids and muscle ammonia detoxification in cirrhosis. *Metab Brain Dis* 2013;28:217-220.
154. Yamamoto M, Iwasa M, Matsumura K, Nakagawa Y, Fujita N, Kobayashi Y, et al. Improvement of regional cerebral blood flow after oral intake of branched-chain amino acids in patients with cirrhosis. *World J Gastroenterol* 2005;11:6792-6799.
155. Plauth M, Egberts EH, Hamster W, Török M, Müller PH, Brand O, et al. Long-term treatment of latent portosystemic encephalopathy with branched-chain amino acids. A double-blind placebo-controlled crossover study. *J Hepatol* 1993;17:308-314.
156. Hayashi S, Aoyagi Y, Fujiwara K, Oka H, Oda T. A randomized controlled trial of branched-chain amino acid (BCAA)-enriched elemental diet (ED-H) for hepatic encephalopathy. *J Gastroenterol Hepatol* 1991;6:191.
157. Horst D, Grace ND, Conn HO, Schiff E, Schenker S, Viteri A, et al. Comparison of dietary protein with an oral, branched chain-enriched amino acid supplement in chronic portal-systemic encephalopathy: a randomized controlled trial. *Hepatology* 1984;4:279-287.
158. Blaha MJ, Martin SS. How do statins work?: changing paradigms with implications for statin allocation. *J Am Coll Cardiol* 2013;62:2392-2394.
159. Pinal-Fernandez I, Casal-Dominguez M, Mammen AL. Statins: pros and cons. *Med Clin (Barc)* 2018;150:398-402.
160. Schierwagen R, Uschner FE, Magdaleno F, Klein S, Trebicka J. Rationale for the use of statins in liver disease. *Am J Physiol Gastrointest Liver Physiol* 2017;312:G407-G412.
161. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015;161:161-172.
162. Marrone G, Russo L, Rosado E, Hide D, García-Cerdeña G, García-Pagán JC, et al. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. *J Hepatol* 2013;58:98-103.
163. Vargas JI, Arrese M, Shah VH, Arab JP. Use of statins in patients with chronic liver disease and cirrhosis: current views and prospects. *Curr Gastroenterol Rep* 2017;19:43.
164. Relja B, Meder F, Wang M, Blaheta R, Henrich D, Marzi I, et al. Simvastatin modulates the adhesion and growth of hepatocellular carcinoma cells via decrease of integrin expression and ROCK. *Int J Oncol* 2011;38:879-885.
165. Dongiovanni P, Petta S, Mannisto V, Mancina RM, Pipitone R, Karja V, et al. Statin use and non-alcoholic steatohepatitis in at risk individuals. *J Hepatol* 2015;63:705-712.
166. Nascimbeni F, Aron-Wisnewsky J, Pais R, Tordjman J, Poitou C, Charlotte F, et al. Statins, antidiabetic medications and liver histology in patients with diabetes with non-alcoholic fatty liver disease. *BMJ Open Gastroenterol* 2016;3:e000075.
167. Ekstedt M, Franzén LE, Mathiesen UL, Holmqvist M, Bodemar G, Kechagias S. Statins in non-alcoholic fatty liver disease and

- chronically elevated liver enzymes: a histopathological follow-up study. *J Hepatol* 2007;47:135-141.
168. Rallidis LS, Drakoulis CK, Parasi AS. Pravastatin in patients with nonalcoholic steatohepatitis: results of a pilot study. *Atherosclerosis* 2004;174:193-196.
169. Hyogo H, Tazuma S, Arihiro K, Iwamoto K, Nabeshima Y, Inoue M, et al. Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia. *Metabolism* 2008;57:1711-1718.
170. Nakahara T, Hyogo H, Kimura Y, Ishitobi T, Arihiro K, Aikata H, et al. Efficacy of rosuvastatin for the treatment of non-alcoholic steatohepatitis with dyslipidemia: an open-label, pilot study. *Hepatol Res* 2012;42:1065-1072.
171. Kargiotis K, Athyros VG, Giouleme O, Katsiki N, Katsiki E, Anagnostis P, et al. Resolution of non-alcoholic steatohepatitis by rosuvastatin monotherapy in patients with metabolic syndrome. *World J Gastroenterol* 2015;21:7860-7868.
172. Hyogo H, Ikegami T, Tokushige K, Hashimoto E, Inui K, Matsuzaki Y, et al. Efficacy of pitavastatin for the treatment of non-alcoholic steatohepatitis with dyslipidemia: an open-label, pilot study. *Hepatol Res* 2011;41:1057-1065.
173. Butt AA, Yan P, Bonilla H, Abou-Samra AB, Shaikh OS, Simon TG, et al. Effect of addition of statins to antiviral therapy in hepatitis C virus-infected persons: results from ERCHIVES. *Hepatology* 2015;62:365-374.
174. Fatima K, Moeed A, Waqar E, Atif AR, Kamran A, Rizvi H, et al. Efficacy of statins in treatment and development of non-alcoholic fatty liver disease and steatohepatitis: a systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2022;46:101816.
175. Zou B, Odden MC, Nguyen MH. Statin use and reduced hepatocellular carcinoma risk in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2022 Feb 11. doi: 10.1016/j.cgh.2022.01.057.
176. Avins AL, Manos MM, Ackerson L, Zhao W, Murphy R, Levin TR, et al. Hepatic effects of lovastatin exposure in patients with liver disease: a retrospective cohort study. *Drug Saf* 2008;31:325-334.
177. Hsiang JC, Wong GL, Tse YK, Wong VW, Yip TC, Chan HL. Statin and the risk of hepatocellular carcinoma and death in a hospital-based hepatitis B-infected population: a propensity score landmark analysis. *J Hepatol* 2015;63:1190-1197.
178. Huang YW, Lee CL, Yang SS, Fu SC, Chen YY, Wang TC, et al. Statins reduce the risk of cirrhosis and its decompensation in chronic hepatitis B patients: a nationwide cohort study. *Am J Gastroenterol* 2016;111:976-985.
179. Simon TG, King LY, Zheng H, Chung RT. Statin use is associated with a reduced risk of fibrosis progression in chronic hepatitis C. *J Hepatol* 2015;62:18-23.
180. Yang YH, Chen WC, Tsan YT, Chen MJ, Shih WT, Tsai YH, et al. Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection. *J Hepatol* 2015;63:1111-1117.
181. Mohanty A, Tate JP, Garcia-Tsao G. Statins are associated with a decreased risk of decompensation and death in veterans with hepatitis C-related compensated cirrhosis. *Gastroenterology* 2016;150:430-40.e1.
182. Abraldes JG, Albillos A, Bañares R, Turnes J, González R, García-Pagán JC, et al. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. *Gastroenterology* 2009;136:1651-1658.
183. Pollo-Flores P, Soldan M, Santos UC, Kunz DG, Mattos DE, da Silva AC, et al. Three months of simvastatin therapy vs. placebo for severe portal hypertension in cirrhosis: a randomized controlled trial. *Dig Liver Dis* 2015;47:957-963.
184. Abraldes JG, Villanueva C, Aracil C, Turnes J, Hernandez-Guerra M, Genesca J, et al. Addition of simvastatin to standard therapy for the prevention of variceal rebleeding does not reduce rebleeding but increases survival in patients with cirrhosis. *Gastroenterology* 2016;150:1160-1170.e3.
185. Kumar S, Grace ND, Qamar AA. Statin use in patients with cirrhosis: a retrospective cohort study. *Dig Dis Sci* 2014;59:1958-1965.
186. Ward NC, Watts GF, Eckel RH. Response by Ward et al to letter regarding article, "Statin Toxicity: Mechanistic Insights and Clinical Implications". *Circ Res* 2019;124:e121-e122.
187. Bhardwaj SS, Chalasani N. Lipid-lowering agents that cause drug-induced hepatotoxicity. *Clin Liver Dis* 2007;11:597-vii.
188. Karahalil B, Hare E, Koç G, Uslu İ, Şentürk K, Özkan Y. Hepatotoxicity associated with statins. *Arh Hig Rada Toksikol* 2017;68:254-260.
189. Björnsson ES. Hepatotoxicity of statins and other lipid-lowering agents. *Liver Int* 2017;37:173-178.
190. Tilg H, Cani PD, Mayer EA. Gut microbiome and liver diseases. *Gut* 2016;65:2035-2044.
191. Fukui H. Leaky gut and gut-liver axis in liver cirrhosis: clinical studies update. *Gut Liver* 2021;15:666-676.
192. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761-1772.
193. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rotteveel J, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007;56:1761-1772.

- tier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 2009;58:1091-1103.
194. Guo S, Al-Sadi R, Said HM, Ma TY. Lipopolysaccharide causes an increase in intestinal tight junction permeability in vitro and in vivo by inducing enterocyte membrane expression and localization of TLR-4 and CD14. *Am J Pathol* 2013;182:375-387.
195. Aron-Wisnewsky J, Warmbrunn MV, Nieuwdorp M, Clément K. Nonalcoholic fatty liver disease: modulating gut microbiota to improve severity? *Gastroenterology* 2020;158:1881-1898.
196. Moya-Pérez A, Neef A, Sanz Y. *Bifidobacterium pseudocatenulatum* CECT 7765 reduces obesity-associated inflammation by restoring the lymphocyte-macrophage balance and gut microbiota structure in high-fat diet-fed mice. *PLoS One* 2015;10:e0126976.
197. Lee NY, Shin MJ, Youn GS, Yoon SJ, Choi YR, Kim HS, et al. *Lactobacillus* attenuates progression of nonalcoholic fatty liver disease by lowering cholesterol and steatosis. *Clin Mol Hepatol* 2021;27:110-124.
198. Rocco A, Sgamato C, Compare D, Cocolli P, Nardone OM, Nardone G. Gut microbes and hepatic encephalopathy: from the old concepts to new perspectives. *Front Cell Dev Biol* 2021;9:748253.
199. Rai R, Saraswat VA, Dhiman RK. Gut microbiota: its role in hepatic encephalopathy. *J Clin Exp Hepatol* 2015;5(Suppl 1):S29-S36.
200. Anand G, Zarrinpar A, Loomba R. Targeting dysbiosis for the treatment of liver disease. *Semin Liver Dis* 2016;36:37-47.
201. Abdel Monem SM. Probiotic therapy in patients with nonalcoholic steatohepatitis in Zagazig University Hospitals. *Euroasian J Hepatogastroenterol* 2017;7:101-106.
202. Wong VW, Won GL, Chim AM, Chu WC, Yeung DK, Li KC, et al. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. *Ann Hepatol* 2013;12:256-262.
203. Nabavi S, Rafrat M, Somi MH, Homayouni-Rad A, Asghari-Jafarabadi M. Effects of probiotic yogurt consumption on metabolic factors in individuals with nonalcoholic fatty liver disease. *J Dairy Sci* 2014;97:7386-7393.
204. Kobyliak N, Abenavoli L, Mykhalchyshyn G, Kononenko L, Boccuto L, Kyriienko D, et al. A multi-strain probiotic reduces the fatty liver index, cytokines and aminotransferase levels in NAFLD patients: evidence from a randomized clinical trial. *J Gastrointestin Liver Dis* 2018;27:41-49.
205. Shavakhi A, Minakari M, Firouzian H, Assali R, Hekmatdoost A, Ferns G. Effect of a probiotic and metformin on liver amino-transferases in non-alcoholic steatohepatitis: a double blind randomized clinical trial. *Int J Prev Med* 2013;4:531-537.
206. Duseja A, Acharya SK, Mehta M, Chhabra S, Shalimar, Rana S, et al. High potency multistrain probiotic improves liver histology in non-alcoholic fatty liver disease (NAFLD): a randomised, double-blind, proof of concept study. *BMJ Open Gastroenterol* 2019;6:e000315.
207. Ahn SB, Jun DW, Kang BK, Lim JH, Lim S, Chung MJ. Randomized, double-blind, placebo-controlled study of a multispecies probiotic mixture in nonalcoholic fatty liver disease. *Sci Rep* 2019;9:5688.
208. Mofidi F, Poustchi H, Yari Z, Nourinayyer B, Merat S, Sharafkhah M, et al. Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial. *Br J Nutr* 2017;117:662-668.
209. Asgharian A, Mohammadi V, Gholi Z, Esmaillzadeh A, Feizi A, Askari G. The effect of synbiotic supplementation on body composition and lipid profile in patients with nafld: a randomized, double blind, placebo-controlled clinical trial study. *Iran Red Crescent Med J* 2017;19:e42902.
210. Manzhali E, Virchenko O, Falalyeyeva T, Beregová T, Stremmel W. Treatment efficacy of a probiotic preparation for non-alcoholic steatohepatitis: a pilot trial. *J Dig Dis* 2017;18:698-703.
211. Sayari S, Neishaboori H, Jameshorani M. Combined effects of synbiotic and sitagliptin versus sitagliptin alone in patients with nonalcoholic fatty liver disease. *Clin Mol Hepatol* 2018;24:331-338.
212. Xiao MW, Lin SX, Shen ZH, Luo WW, Wang XY. Systematic review with meta-analysis: the effects of probiotics in nonalcoholic fatty liver disease. *Gastroenterol Res Pract* 2019;2019:1484598.
213. Dhiman RK, Rana B, Agrawal S, Garg A, Chopra M, Thumbaru KK, et al. Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* 2014;147:1327-37.e3.
214. Lunia MK, Sharma BC, Sharma P, Sachdeva S, Srivastava S. Probiotics prevent hepatic encephalopathy in patients with cirrhosis: a randomized controlled trial. *Clin Gastroenterol Hepatol* 2014;12:1003-1008.e1.
215. Dalal R, McGee RG, Riordan SM, Webster AC. Probiotics for people with hepatic encephalopathy. *Cochrane Database Syst Rev* 2017;2:CD008716.
216. Bafeta A, Koh M, Riveros C, Ravaud P. Harms reporting in randomized controlled trials of interventions aimed at modifying microbiota: a systematic review. *Ann Intern Med* 2018;169:240-

- 247.
217. Didari T, Solki S, Mozaffari S, Nikfar S, Abdollahi M. A systematic review of the safety of probiotics. *Expert Opin Drug Saf* 2014;13:227-239.
218. Goldenberg JZ, Lytvyn L, Steurich J, Parkin P, Mahant S, Johnston BC. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2015;(12):CD004827.
219. Zingg JM. Vitamin E: an overview of major research directions. *Mol Aspects Med* 2007;28:400-422.
220. Ahsan H, Ahad A, Iqbal J, Siddiqui WA. Pharmacological potential of tocotrienols: a review. *Nutr Metab (Lond)* 2014;11:52.
221. Tabei SM, Fakher S, Djalali M, Javanbakht MH, Zarei M, Derakhshanian H, et al. Effect of vitamins A, E, C and omega-3 fatty acids supplementation on the level of catalase and superoxide dismutase activities in streptozotocin-induced diabetic rats. *Bratisl Lek Listy* 2015;116:115-118.
222. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic Biol Med* 2014;72:76-90.
223. Jin X, Song L, Liu X, Chen M, Li Z, Cheng L, et al. Protective efficacy of vitamins C and E on p,p'-DDT-induced cytotoxicity via the ROS-mediated mitochondrial pathway and NF-κB/FasL pathway. *PLoS One* 2014;9:e113257.
224. Shen XH, Cheng WF, Li XH, Sun JQ, Li F, Ma L, et al. Effects of dietary supplementation with vitamin E and selenium on rat hepatic stellate cell apoptosis. *World J Gastroenterol* 2005;11:4957-4961.
225. Masarone M, Rosato V, Dallio M, Gravina AG, Aglitti A, Loguerico C, et al. Role of oxidative stress in pathophysiology of nonalcoholic fatty liver disease. *Oxid Med Cell Longev* 2018;2018:9547613.
226. Magosso E, Ansari MA, Gopalan Y, Shuaib IL, Wong JW, Khan NA, et al. Tocotrienols for normalisation of hepatic echogenic response in nonalcoholic fatty liver: a randomised placebo-controlled clinical trial. *Nutr J* 2013;12:166.
227. Fouda A, Abdelaziz AE, Hussien M, Ali AA, Abdelkawy KS, Elbarbry F. A randomized controlled trial comparing the effects of vitamin E, ursodeoxycholic acid and pentoxifylline on Egyptian non-alcoholic steatohepatitis patients. *Eur Rev Med Pharmacol Sci* 2021;25:7449-7459.
228. Kedarisetty CK, Bhardwaj A, Kumar G, Rastogi A, Bihari C, Kumar M, et al. Efficacy of combining pentoxifylline and vitamin E versus vitamin E alone in non-alcoholic steatohepatitis-a randomized pilot study. *Indian J Gastroenterol* 2021;40:41-49.
- 49.
229. Polyzos SA, Kountouras J, Mantzoros CS, Polymerou V, Katsinelos P. Effects of combined low-dose spironolactone plus vitamin E vs vitamin E monotherapy on insulin resistance, non-invasive indices of steatosis and fibrosis, and adipokine levels in non-alcoholic fatty liver disease: a randomized controlled trial. *Diabetes Obes Metab* 2017;19:1805-1809.
230. Pervez MA, Khan DA, Slehra AUR, Ijaz A. Delta-tocotrienol supplementation improves biochemical markers of hepatocellular injury and steatosis in patients with nonalcoholic fatty liver disease: A randomized, placebo-controlled trial. *Complement Ther Med* 2020;52:102494.
231. Ekhlaei G, Kolahdouz Mohammadi R, Agah S, Zarrati M, Hosseini AF, Arabshahi SS, et al. Do symbiotic and vitamin E supplementation have favorite effects in nonalcoholic fatty liver disease? A randomized, double-blind, placebo-controlled trial. *J Res Med Sci* 2016;21:106.
232. Aller R, Izaola O, Gómez S, Tafur C, González G, Berroa E, et al. Effect of silymarin plus vitamin E in patients with non-alcoholic fatty liver disease. A randomized clinical pilot study. *Eur Rev Med Pharmacol Sci* 2015;19:3118-3124.
233. Harrison SA, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2003;98:2485-2490.
234. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67:328-357.
235. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia* 2016;59:1121-1140.
236. Chitturi S, Wong VW, Chan WK, Wong GL, Wong SK, Sollano J, et al. The Asia-Pacific working party on non-alcoholic fatty liver disease guidelines 2017-part 2: management and special groups. *J Gastroenterol Hepatol* 2018;33:86-98.
237. Bril F, Biernacki DM, Kalavalapalli S, Lomonaco R, Subbarayan SK, Lai J, et al. Role of vitamin E for nonalcoholic steatohepatitis in patients with type 2 diabetes: a randomized controlled trial. *Diabetes Care* 2019;42:1481-1488.
238. Vadalis A, Antza C, Bakaloudi DR, Doundoulakis I, Kalopitas G, Samara M, et al. Systematic review with meta-analysis: the effect of vitamin E supplementation in adult patients with

- non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2021;36:311-319.
239. Said A, Akhter A. Meta-analysis of randomized controlled trials of pharmacologic agents in non-alcoholic steatohepatitis. *Ann Hepatol* 2017;16:538-547.
240. Majzoub AM, Nayfeh T, Barnard A, Munaganuru N, Dave S, Singh S, et al. Systematic review with network meta-analysis: comparative efficacy of pharmacologic therapies for fibrosis improvement and resolution of NASH. *Aliment Pharmacol Ther* 2021;54:880-889.
241. Choi J, Ou JH. Mechanisms of liver injury. III. Oxidative stress in the pathogenesis of hepatitis C virus. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G847-G851.
242. Jain SK, Pemberton PW, Smith A, McMahon RF, Burrows PC, Aboutwerat A, et al. Oxidative stress in chronic hepatitis C: not just a feature of late stage disease. *J Hepatol* 2002;36:805-811.
243. Chuma M, Hige S, Nakanishi M, Ogawa K, Natsuizaka M, Yamamoto Y, et al. 8-Hydroxy-2'-deoxy-guanosine is a risk factor for development of hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2008;23:1431-1436.
244. von Herbay A, Stahl W, Niederau C, Sies H. Vitamin E improves the aminotransferase status of patients suffering from viral hepatitis C: a randomized, double-blind, placebo-controlled study. *Free Radic Res* 1997;27:599-605.
245. Malaguarnera M, Motta M, Vacante M, Malaguarnera G, Caraci F, Nunnari G, et al. Silybin-vitamin E-phospholipids complex reduces liver fibrosis in patients with chronic hepatitis C treated with pegylated interferon  $\alpha$  and ribavirin. *Am J Transl Res* 2015;7:2510-2518.
246. Marotta F, Yoshida C, Barreto R, Naito Y, Packer L. Oxidative-inflammatory damage in cirrhosis: effect of vitamin E and a fermented papaya preparation. *J Gastroenterol Hepatol* 2007;22:697-703.
247. Idéo G, Bellobuono A, Tempini S, Mondazzi L, Airoldi A, Benetti G, et al. Antioxidant drugs combined with alpha-interferon in chronic hepatitis C not responsive to alpha-interferon alone: a randomized, multicentre study. *Eur J Gastroenterol Hepatol* 1999;11:1203-1207.
248. Groenbaek K, Friis H, Hansen M, Ring-Larsen H, Krarup HB. The effect of antioxidant supplementation on hepatitis C viral load, transaminases and oxidative status: a randomized trial among chronic hepatitis C virus-infected patients. *Eur J Gastroenterol Hepatol* 2006;18:985-989.
249. Bunchorntavakul C, Wootthananon T, Atsawarungruangkit A. Effects of vitamin E on chronic hepatitis C genotype 3: a randomized, double-blind, placebo-controlled study. *J Med Assoc Thai* 2014;97 Suppl 11:S31-S40.
250. Look MP, Gerard A, Rao GS, Sudhop T, Fischer HP, Sauerbruch T, et al. Interferon/antioxidant combination therapy for chronic hepatitis C-a controlled pilot trial. *Antiviral Res* 1999;43:113-122.
251. Andreone P, Fiorino S, Cursaro C, Gramenzi A, Margotti M, Di Giannarino L, et al. Vitamin E as treatment for chronic hepatitis B: results of a randomized controlled pilot trial. *Antiviral Res* 2001;49:75-81.
252. Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* 2005;142:37-46.
253. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 2007;297:842-857.
254. Klein EA, Thompson IM Jr, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2011;306:1549-1556.
255. Schürks M, Glynn RJ, Rist PM, Tzourio C, Kurth T. Effects of vitamin E on stroke subtypes: meta-analysis of randomised controlled trials. *BMJ* 2010;341:c5702.
256. Jiang ZG, Feldbrügge L, Tapper EB, Popov Y, Ghaziani T, Afshar N, et al. Aspirin use is associated with lower indices of liver fibrosis among adults in the United States. *Aliment Pharmacol Ther* 2016;43:734-743.
257. Yoshida S, Ikenaga N, Liu SB, Peng ZW, Chung J, Sverdlov DY, et al. Extrahepatic platelet-derived growth factor- $\beta$ , delivered by platelets, promotes activation of hepatic stellate cells and biliary fibrosis in mice. *Gastroenterology* 2014;147:1378-1392.
258. Wolf MJ, Adili A, Piotrowitz K, Abdullah Z, Boege Y, Stemmer K, et al. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell* 2014;26:549-564.
259. Malehmir M, Pfister D, Gallage S, Szydlowska M, Inverso D, Kotsiliri E, et al. Platelet GPIba is a mediator and potential interventional target for NASH and subsequent liver cancer. *Nat Med* 2019;25:641-655.
260. Wang T, Fu X, Jin T, Zhang L, Liu B, Wu Y, et al. Aspirin targets P4HA2 through inhibiting NF- $\kappa$ B and LMCD1-AS1/let-7g to inhibit tumour growth and collagen deposition in hepatocellular

- carcinoma. *EBioMedicine* 2019;45:168-180.
261. Iannacone M, Sitia G, Narvaiza I, Ruggeri ZM, Guidotti LG. Antiplatelet drug therapy moderates immune-mediated liver disease and inhibits viral clearance in mice infected with a replication-deficient adenovirus. *Clin Vaccine Immunol* 2007;14:1532-1535.
262. Shen H, Shahzad G, Jawairia M, Bostick RM, Mustacchia P. Association between aspirin use and the prevalence of nonalcoholic fatty liver disease: a cross-sectional study from the third national health and nutrition examination survey. *Aliment Pharmacol Ther* 2014;40:1066-1073.
263. Simon TG, Henson J, Osganian S, Masia R, Chan AT, Chung RT, et al. Daily aspirin use associated with reduced risk for fibrosis progression in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2019;17:2776-2784.e4.
264. Petrick JL, Sahasrabuddhe VV, Chan AT, Alavanja MC, Beane Freeman LE, Buring JE, et al. NSAID use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: the liver cancer pooling project. *Cancer Prev Res (Phila)* 2015;8:1156-1162.
265. Simon TG, Ma Y, Ludvigsson JF, Chong DQ, Giovannucci EL, Fuchs CS, et al. Association between aspirin use and risk of hepatocellular carcinoma. *JAMA Oncol* 2018;4:1683-1690.
266. Li X, Wu S, Yu Y. Aspirin use and the incidence of hepatocellular carcinoma in patients with hepatitis B virus or hepatitis C virus infection: a meta-analysis of cohort studies. *Front Med (Lausanne)* 2021;7:569759.
267. Choi WM, Kim HJ, Jo AJ, Choi SH, Han S, Ko MJ, et al. Association of aspirin and statin use with the risk of liver cancer in chronic hepatitis B: a nationwide population-based study. *Liver Int* 2021;41:2777-2785.
268. Lee TY, Hsu YC, Tseng HC, Lin JT, Wu MS, Wu CY. Association of daily aspirin therapy with hepatocellular carcinoma risk in patients with chronic hepatitis c virus infection. *Clin Gastroenterol Hepatol* 2020;18:2784-2792.e7.
269. Lee TY, Hsu YC, Tseng HC, Yu SH, Lin JT, Wu MS, et al. Association of daily aspirin therapy with risk of hepatocellular carcinoma in patients with chronic hepatitis B. *JAMA Intern Med* 2019;179:633-640.
270. Liao YH, Hsu RJ, Wang TH, Wu CT, Huang SY, Hsu CY, et al. Aspirin decreases hepatocellular carcinoma risk in hepatitis C virus carriers: a nationwide cohort study. *BMC Gastroenterol* 2020;20:6.
271. Hui VW, Yip TC, Wong VW, Tse YK, Chan HL, Lui GC, et al. Aspirin reduces the incidence of hepatocellular carcinoma in patients with chronic hepatitis b receiving oral nucleos(t)ide analog. *Clin Transl Gastroenterol* 2021;12:e00324.
272. Lee PC, Yeh CM, Hu YW, Chen CC, Liu CJ, Su CW, et al. Antiplatelet therapy is associated with a better prognosis for patients with hepatitis B virus-related hepatocellular carcinoma after liver resection. *Ann Surg Oncol* 2016;23(Suppl 5):874-883.
273. Li JH, Wang Y, Xie XY, Yin X, Zhang L, Chen RX, et al. Aspirin in combination with TACE in treatment of unresectable HCC: a matched-pairs analysis. *Am J Cancer Res* 2016;6:2109-2116.
274. Young SH, Chau GY, Lee IC, Yeh YC, Chao Y, Huo TI, et al. Aspirin is associated with low recurrent risk in hepatitis B virus-related hepatocellular carcinoma patients after curative resection. *J Formos Med Assoc* 2020;119(1 Pt 2):218-229.
275. Tan RZH, Lockart I, Abdel Shaheed C, Danta M. Systematic review with meta-analysis: the effects of non-steroidal anti-inflammatory drugs and anti-platelet therapy on the incidence and recurrence of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2021;54:356-367.
276. Lee M, Chung GE, Lee JH, Oh S, Nam JY, Chang Y, et al. Antiplatelet therapy and the risk of hepatocellular carcinoma in chronic hepatitis B patients on antiviral treatment. *Hepatology* 2017;66:1556-1569.
277. Hanai T, Shiraki M, Nishimura K, Ohnishi S, Imai K, Suetsugu A, et al. Sarcopenia impairs prognosis of patients with liver cirrhosis. *Nutrition* 2015;31:193-199.
278. Lee JI, Lee HW, Lee KS, Lee HS, Park JY. Effects of statin use on the development and progression of nonalcoholic fatty liver disease: a nationwide nested case-control study. *Am J Gastroenterol* 2021;116:116-124.
279. Sanyal AJ, Mofrad PS, Contos MJ, Sargeant C, Luketic VA, Sterling RK, et al. A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2004;2:1107-1115.
280. Ersöz G, Günşar F, Karasu Z, Akay S, Batur Y, Akarca US. Management of fatty liver disease with vitamin E and C compared to ursodeoxycholic acid treatment. *Turk J Gastroenterol* 2005;16:124-128.